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Relative Resistance of Free and Encysted[1] Larvae of the Golden Nematode *Heterodera rostochiensis* Wollenweber to D-D Mixture and Hot Water

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It frequently has been suggested that the efficacy of a fumigant used to kill the golden nematode of potatoes, *Heterodera rostochiensis* Wollenweber might be increased by application to the soil, before fumigation, of a substance which would cause the larvae to emigrate from the cysts. This suggestion is based on the assumption, unsupported by published data, that the fumigant would have considerably greater toxic effect to free or emigrated larvae than to encysted larvae. Allen and Raski (1950) reported that larvae of the root knot nematode and encysted eggs of the sugar beet nematode are about equally susceptible to dichloropropene (D-D) mixture, while ethylene dibromide under conditions lethal to the former is ineffective against the latter. In this paper, data are reported on the relative toxicity to encysted larvae and free larvae of D-D mixture at a number of concentrations, and to hot water at several temperatures. D-D mixture was used because it is the most promising chemical for killing golden nematodes in the soil.

GENERAL METHODS

It was necessary to devise a method for handling larvae in the hot water and D-D treatments. Large numbers of free larvae were obtained by placing cysts in potato root leachings. The larvae were separated from the cysts by screening. The concentration of larvae per volume of suspension was determined. It was then possible to obtain approximately equal numbers of larvae by removing equal volumes of suspension with a pipette while the suspension was being stirred.

The larval suspension was pipetted into a filter paper in a glass funnel. The water passed through the paper leaving the larvae in the folded filter paper. The larvae were then washed to the point of the paper cone with sterile water. While the paper was still moist, it was folded from the sides, top and bottom and placed in small cotton or nylon bags approximately three inches square. Preliminary tests showed that similar results were obtained in nylon or cotton bags. Nylon bags are preferred because of their greater durability. The bags were folded twice and a small rubber band wound around them.

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[1] In this paper the term "encysted" refers to larvae enclosed in the dead, matured body of a female.

The writers are indebted to Miss Joyce von Mechow for making nematode counts reported in this paper.

A number of tests showed that approximately 90 per cent of the larvae placed on the filter paper could be recovered when the larvae were left overnight in the folded filter paper and from 40 to 50 per cent when the larvae were left for one week. When the larvae in the folded filter papers were left for one week in moist soil only approximately 15 per cent were recovered. The reasons for the different recovery rates are not known. Data from additional tests indicated that a lethal treatment with hot water for five minutes at 131°F. did not decrease the total number of larvae recovered from filter paper.

RESISTANCE OF FREE LARVAE TO HOT WATER

Triffitt and Hurst (1935), after testing the efficacy of five-minute hot water treatments at various temperatures, concluded that 130°F. was the lowest temperature at which all encysted larvae were killed. These findings have been confirmed repeatedly by the workers at this laboratory. A preliminary test was conducted to determine the resistance of free larvae to several hot water treatments. Larvae, confined in filter paper, were immersed in a water bath at temperatures of 110, 115, 120, 125 and 130° F. for five minutes. After treatment the larvae were transferred from the filter paper into petri dishes. There were two replications of each treatment.

After 26 days the larvae were examined for viability. Viability was determined not only by appearance but also by motility. At the suggestion of Miss Edna M. Buhrer of the Bureau of Nematology of the U. S. Department of Agriculture the unhealthy-appearing larvae which were not moving were touched with a bamboo needle at the nerve ring, and those in which voluntary movement occurred were counted as living.

All larvae were killed when treated at 130° and 125°F. and only one living larva was found after treatment at 120°F. (Table 1). Free larvae thus appear more susceptible to hot water treatment than encysted larvae. A direct comparison, however, was not made.

TABLE 1

SURVIVAL OF LARVAE FOLLOWING 5-MINUTE TREATMENTS IN HOT WATER

Treatment °F.	Total number larvae examined	Per cent living after 25 days
130	7,156	0
125	5,344	0
120	3,152	0.3
115	4,036	2.5
110	6,612	32.1
Control	1,620	95.0

RELATIVE RESISTANCE OF FREE AND ENCYSTED LARVAE TO HOT WATER TREATMENTS

Viabilities Assayed by Examining Free Larvae and by Counting the Emergence of Encysted Larvae in Potato Root Leachings. Cotton or nylon bags containing 2,000 larvae or 500 cysts in the filter papers were soaked two hours to facilitate wetting of the bags and contents. Larvae and cysts were treated simultaneously by immersing one bag with larvae and one bag with cysts in a water bath accurate to approximately ± 1°F. Each treatment was replicated eight times. The untreated checks, eight bags of larvae and eight bags of cysts, were immersed in water from 60-70°F. for five minutes.

All filter papers were removed from the bags. The cysts were washed into petri dishes containing 35-40 ml of potato leachings and the larvae into petri dishes with 35-40 ml sterile water. The petri dishes were placed in the dark at 75°F.

Viability of free larvae was tested in each replication by examination of 100 larvae chosen at random. The examinations were made three weeks after treatment to facilitate the detection of the dead larvae.

Viability of encysted larvae was tested by observation of the emergence of larvae from treated cysts. Because different ways of determining viability were employed it was not possible to compare directly the figures denoting viability of free larvae with those denoting viability of encysted larvae. Hence an indirect method of comparison was used.

The viability of free larvae, determined by direct examination, was compared with the viability of non-treated free larvae, and the relationship expressed as a percentage. Similarly, the viability of encysted larvae, measuring the number of larvae emerging in potato root leachings, was compared with the viability of untreated encysted larvae, and the relationship expressed as a percentage. The corresponding percentage figures for a given treatment were considered a valid measure of the relative resistance of free and encysted larvae to the treatments.

A summary of the results obtained in two separate experiments show that the free larvae were easier to kill with hot water than were encysted larvae (Table 2). A complete kill of free larvae was obtained at 125°F. and a complete kill of encysted larvae at 131°F.

TABLE 2

SURVIVAL OF ENCYSTED AND FREE LARVAE FOLLOWING 5-MINUTE TREATMENTS IN HOT WATER

	Larvae surviving			
	Encysted larvae		Free larvae	
Treatment °F.	No. larvae emerging	% of control	No. viable larvae	% of control
131	0	0	0	0
125	830	14	0	0*
120	4052	67	193	13*
Control	6041	----	1527	----

*Significantly less than encysted larvae at odds of greater than 99-1.

Viabilities Assayed by Infection of Potato Roots. A final criterion of viability is whether or not larvae are capable of producing new individuals on potato roots. This method of establishing viability was used in a third experiment involving hot water treatment of encysted and free larvae.

One thousand cysts were opened and the contents examined. It was found that there were approximately 14 viable larvae per cyst. In order to obtain equivalent inocula 2500 cysts and 35,000 larvae, respectively, were included in a single replication. Treatments were made in the same manner as was described previously. Untreated checks, and five-minute treatments of encysted and free larvae at 130, 120, 115, 110 and 100°F. were included in this experiment. Eight replications were used.

After treatment, cysts and larvae were removed from the filter papers and washed into four-inch pots containing sterile soil. One potato seed piece was added to each pot. Approximately one-half inch of sterile soil was sprinkled on the surface of the soil to which the inocula were added to prevent splashing of larvae or cysts between pots.

The majority of the potato roots were harvested when the immature females had begun to turn yellow. A determination was made in the laboratory of the number of immature females per gram of root. The free larvae were

TABLE 3

SURVIVAL OF ENCYSTED AND FREE LARVAE IN HOT WATER

Degrees Fahrenheit for 5 minutes	No. of immature females per gram of root		
	Encysted larvae	Free larvae	Encysted larvae/ free larvae
130	1	0	--
120	377	1*	377
115	1024	260*	3.9
110	2197	844*	2.6
100	1991	1124*	1.8
Check	1920	1297*	1.5

found to be more easily killed than were encysted larvae (Table 3). All of the free larvae and practically all of the encysted larvae were killed by the 130° treatment. In the case of the remaining treatments and the untreated check, the numbers of immature females per gram of root resulting from free larvae added to the soil were significantly less than those resulting from encysted larvae. However, as the temperatures were lowered the relative survival of the treated free larvae as compared with the treated encysted larvae became greater. In this relationship the untreated check is practically the same as the 100° treatments.

TREATMENT OF FREE AND ENCYSTED LARVAE WITH DICHLOROPROPENE-DICHLOROPROPANE MIXTURE

Viabilities Assayed by Examining Free Larvae and by Counting the Emergence of Encysted Larvae in Potato Root Leachings. D-D mixture was used to test the relative resistance of free and encysted larvae to a volatile chemical in the soil. The concentration of D-D mixture was varied by placing free and encysted larvae at various distances, both vertically and horizontally, from the point of injection of the chemical. The encysted and free larvae were prepared for treatment in the same manner as those for the first hot water treatments. Approximately 500 cysts and 2,000 free larvae were placed in each of 42 filter papers. The 84 filter papers were arranged in pairs at random, one of each pair containing encysted larvae and one containing free larvae. Six of these pairs were placed in non-infested soil and left as untreated checks. The remaining 36 were placed in a metal container of non-infested soil. The container was square with rounded corners, 15 by 15 inches at the bottom, 18.5 inches by 18.5 inches at the top and 10.5 inches deep.

The technique employed for placing bags in the soil was that used by Lear (1951). Twelve of the 36 pairs of filter papers were placed in the soil one and one-half inches from the bottom of the tub, twelve pairs five inches from the bottom of the tub, and twelve pairs seven and one-half inches from the bottom of the tub or three inches from the top of the soil. Four of the twelve filter papers at each level were placed two inches horizontally from the center of the tub, four were placed five inches from the center, and four

*Significantly less than encysted larvae at odds of greater than 99-1.

eight inches from the center. In each case the samples were equally placed around the circumference of a circle, the center of which was the center of the tub. One milliliter of D-D mixture was added to the soil in the center of the tub, three inches from the surface. No surface seal was used. The pairs of papers were placed so that one would not interfere with the diffusion of D-D towards any other.

One week after the D-D injection, the filter papers were removed from the treated and the untreated soil. The free larvae were washed from the filter papers into petri dishes with 35 ml. of sterile water, and the cysts were washed from the filter papers into petri dishes with 35 ml. of fresh potato root leachings.

After three weeks the petri dishes with free larvae were examined for percentage of viable larvae, and the petri dishes with cysts were examined for the number of larvae emerged. The viability of free larvae was compared with that of the free larval checks. Larval emergence from treated cysts was compared with that from untreated cysts.

When the results of three experiments were combined the survival of the treated encysted larvae was 61.6 per cent of the untreated encysted larvae while the survival of the treated free larvae was 50.3 per cent of the survival of the untreated free larvae. The difference between the two percentage figures was mathematically significant at odds greater than 99-1. A complete kill of either the encysted or free larvae was not obtained at any location in the container. A 99.5 per cent kill of free larvae two inches from the point of injection of the D-D mixture was obtained. The kill of encysted larvae at this location was 93 per cent.

Viabilities Assayed by Infection of Potato Roots. In another experiment, in which encysted and free larvae were exposed to various concentrations of D-D mixture in the manner previously described, viabilities were assayed by the production of new individuals on potato roots. The procedure used in obtaining the numbers of immature females per gram of root was the same as that described for the hot water treatments. The data from this experiment (Table 4) show that under the conditions of this experiment the resistance of free larvae to D-D mixture is considerably less than the resistance of encysted larvae to various concentrations of this chemical. Again complete eradication of encysted or free larvae was not accomplished at any location. In this experiment the number of immature females on the potato roots grown in pots receiving non-treated encysted and free larval inoculum were approxi-

TABLE 4

RESISTANCE OF ENCYSTED AND FREE LARVAE TO 1,3 DICHLOROPROPENE 1,2 DICHLOROPROPANE MIXTURE

Treatments	Immature females per gram of root	
	Encysted larvae	Free larvae
Not treated	633	651
D-D mixture	490	78*

mately equal. The percentage kill of the encysted larvae was very low. In these experiments an attempt was made to obtain partial kills of both en-

*Significantly less than encysted larvae at odds of more than 99-1.

cysted and free larvae rather than high percentage kills of either of them. The resistance of free larvae to D-D mixture when compared with the resistance of encysted larvae appeared to be greater when the formation of new individuals was used as a criterion of viability than when the examination of free larvae, and the emergence of larvae from cysts were used as viability criteria. It is possible that free larvae receiving low concentrations of D-D mixture appear healthy but are unable to infect potato roots. The unprotected free larvae are probably more susceptible to attack by other soil organisms than are encysted larvae.

DISCUSSION

The data presented in this paper indicate that free larvae of the golden nematode are less resistant to treatment with D-D mixture than are encysted larvae.

A chemical added to the soil to stimulate the emergence of larvae from cysts would therefore seem to be of value in increasing the kill of this pest in the soil by fumigation. Furthermore, as has been suggested by Oostenbrink (1950) and Lownsbery (1951) the application of a stimulating agent to soil heavily-infested with the golden nematode might shorten the years of crop rotation necessary between profitable crops of potatoes. However, to be effective a stimulant must remain unaltered in the soil for long periods of time as only a small percentage of the larvae emerge when a stimulant is first applied. In addition, a practical way must be found to thoroughly mix the stimulant with the soil in potato fields. Up to the present time only volatile chemicals have been mixed successfully in the soil under field conditions. A volatile chemical would not remain in the soil for a long period of time unless it was a slowly volatilizing solid.

It is possible that a chemical will be found which at very low concentrations will stimulate larvae to emerge from cysts, will remain in the soil, and can be thoroughly mixed with the soil. At the present time the probabilities of finding such a chemical appear to be small. The finding of a volatile chemical more lethal to encysted larvae and more effective methods of applying chemicals appear to hold more promise for obtaining a higher percentage kill of golden nematodes in the soil.

SUMMARY

A method was developed for handling free larvae of the golden nematode experimentally in aqueous suspensions and in soil.

In one set of experiments the viability of the free larvae was determined by direct examination, and the viability of encysted larvae by emigration from cysts under the influence of potato root excretion. In the other group of experiments the viability of both encysted and free larvae was tested by their production of new individuals on potato roots.

Free larvae were found to be more easily killed by hot water than were encysted larvae. Likewise D-D mixture was more toxic to free larvae in soil than encysted larvae when tested at nine distances from the injection point of the chemical.

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Studies on the Helminth Fauna of Alaska. XIV. Some Cestode Parasites of the Aleutian Teal (*Anas crecca* L.) With the Description of *Diorchis longiovum* n. sp.

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The Aleutian teal (*Anas crecca nimia* Friedmann[2]) has been relatively unavailable for helminth investigations by American workers because its range in North America is restricted to the western-most Aleutian Islands.

During some parasitological studies on Amchitka, Aleutian Islands, Alaska, in May and early June 1952, in connection with sea otter mortality, the writer had the opportunity to collect 20 adult Aleutian teal. These birds, consisting of 16 males (average weight, 392 grams) and 4 females (average weight, 353 grams) were taken at the beginning of the nesting season. Autopsies revealed that 16 (80%) of these ducks were parasitized by cestodes. All infections were considered to be relatively light—the numbers of cestodes recovered ranged from one to 25. Subsequent taxonomic study disclosed that the cestodes represented three genera and four species; viz., *Hymenolepis collaris* (Batsch, 1786); *Fimbriaria fasciolaris* (Pallas, 1871); *Diorchis acuminata* Clerc, 1902; and a new species, herein described, of the genus *Diorchis*.

H. collaris was found most frequently, occurring in 10 of the 16 parasitized ducks. This cestode comprised the only species present in 7 of the 10 birds and occurred together with another species in three—once with *D. acuminata* and twice with *Diorchis* n. sp.

F. fasciolaris was found in only one of the ducks which, at the same time, harbored the new species of *Diorchis*.

D. acuminata was found in two birds, once with *H. collaris* and once with the new species.

The cestode herein described occurred as the only species in four of the ducks and together with other cestodes as indicated above, in four.

No other helminth parasites were obtained from the Aleutian teal examined in this study.

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[2]This host has been identified by Dr. H. Friedmann, curator, Division of Birds, U. S. National Museum, Washington, D. C.

Diorchis longiovum n. sp.
(Figs. 1-5)

DIAGNOSIS: (Hymenolepididae.) Length of strobila about 85-100 mm.; maximum width 1.2 mm. attained in post-mature proglottids. Scolex 224 x 308 μ. Suckers 90 x 135 μ. Suckers spinose. Pocket of sucker covered with minute spines. Margin of sucker beset with coarse spines. Evaginated rostellum 279 μ in length from base and 72 μ in diameter at base. Rostellum provided with a single row of 10 hooks, 57 μ in length. Strobila 150 μ wide immediately posterior to base of scolex. Genital pores unilateral and dextral. Genital ducts between dorsal and ventral excretory canals. Muscular cirrus sac averages 530 μ in length by 39 μ in width. Cirrus sac extends aporad to ventral excretory canal. External seminal vesicle well developed, spherical and lies ventrally upon aporal end of cirrus sac in late-mature proglottids. Cirrus sparsely armed with coarse spines at proximal end for a distance approximately one-third the length of cirrus when extended. Testes, two in number, subspherical to ovoid, about 126 μ in diameter in mature proglottids. One testis occurs poral and the other aporal to ovary and vitelline gland. Poral testis lies in a plane slightly more ventral than aporal testis. Ovary irregular to trilobate, located in middle of proglottid. Vitelline gland ovoid to irregular in shape, lies ventral to and on posterior surface of ovary. Vagina lies ventral and slightly posterior to cirrus sac. Ovoid seminal receptacle lies dorsal to poral ventral excretory canal between cirrus sac and poral testis. Uterus extends as an irregular tube transversely across middle of proglottid and develops by enlargement, becoming sacculate and filling entire proglottid when completely gravid. Eggs of elongate spindle shape. Spindles drawn out into long filamentous processes. Eggs average (from tip to tip of spindles) 96 μ in length by 15 μ in maximum diameter. Embryo measures 52.5 x 11 μ. Embryo hooks 7.5 μ in length. Ventral longitudinal excretory canals 45 μ in diameter; dorsal canals 9 μ in diameter.

HOST: *Anas crecca* L.

LOCALITY: Amchitka, Aleutian Islands, Alaska.

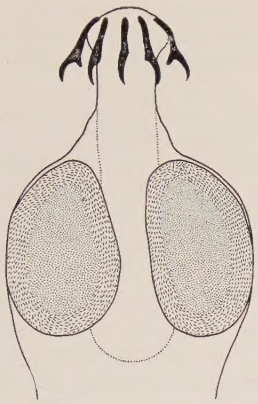
HABITAT: Small intestine.

TYPE: One slide, No. 47860, containing an entire specimen, has been deposited in the Helminthological Collection of the U. S. National Museum, Washington, D. C.

PARATYPE: One slide, No. 47861.

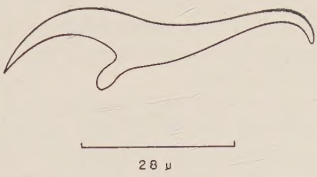
DISCUSSION: Schultz (1940) reviewed the genus *Diorchis* and compiled a list of 23 species belonging to this group. Two species previously described by Johri (1939), *D. alvedea* from *Streptopelia orientalis* (Latham, 1790) and *D. chalcophapsi* from *Chalcophaps indica* (Linnaeus, 1758), apparently did not come to the attention of Schultz in time to be included in his treatment of the genus. Insofar as the writer is aware, only three additional species have been described since the work of Schultz. These are *D. anomala* Schmelz, 1941 (from an anseriform bird), *D. ralli* Jones, 1944 (from Gruiformes), and *D. reynoldsi* Jones, 1944 (from a mammal). The species herein described brings the total number in this genus to 29.

SYNONOMY: Of the 29 species assigned to the genus *Diorchis*, only one, *D. reynoldsi*, is reportedly found in a mammalian host (*Blarina brevicauda* in Virginia). Rausch and Kuns (1950), in their studies on North American shrew cestodes, did not find this species in *Blarina* nor any other shrew species. They stated that, "It [*D. reynoldsi*] appears to have a restricted distribution." In a discussion of shrew cestodes in the Hocking County area



100 μ

1

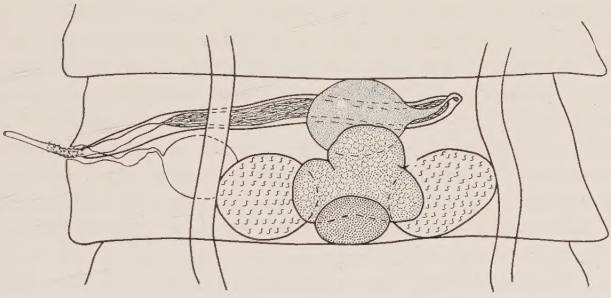


28 μ

2

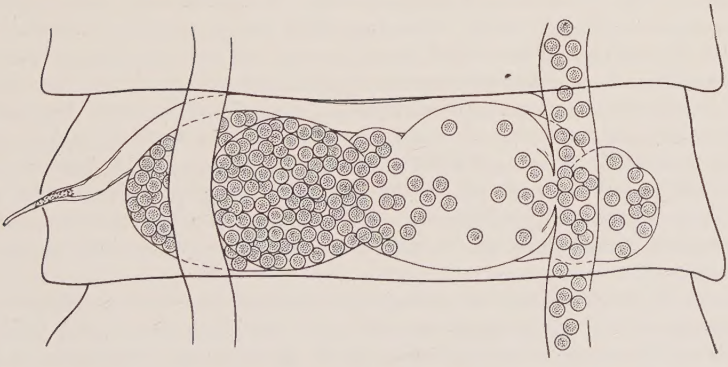
25 μ

3



280 μ

4



700 μ

5

Morphological details of *Diorchis longiovum* n. sp. Fig. 1. Scolex. Fig. 2. Rostellar hook. Fig. 3. Egg. Fig. 4. Mature proglottid (ventral view). Fig. 5. Early gravid proglottid showing entry of eggs into lumen of ventral exeretory canal from uterus through abnormal passage formed between these organs.

of Ohio, Oswald (1951) observed that, ". . . . *Diorchis reynoldsi* Jones, 1944, and *Protogynella blarinae* Jones, 1943, are of common occurrence in *Blarina brevicauda* in this area."

Examination of the type specimen of *D. reynoldsi,* made possible through the kindness of Dr. E. W. Price, Assistant Chief, Zoological Division, Agricultural Research Center, Bureau of Animal Industry, revealed the presence of three testes rather than two as reported by Jones in the original diagnosis. Inasmuch as testes number constitutes a generic character in the family Hymenolepididae, removal of this species from the genus *Diorchis* and assignment to the genus *Hymenolepis* is recommended. This recommendation is substantiated on the basis of the rostellar armature in this species (more than 100 minute hooks) which is more characteristic of the genus *Hymenolepis* than of the genus to which it has been assigned. The mascerated condition of the type specimen precludes much detailed analysis of specific characters necessary to differentiate it from other closely related species of the genus *Hymenolepis;* therefore determination of its specific status must remain questionable at present, pending the study of additional material in a more favorable condition.

Long and Wiggins (1939) described a species of the genus *Diorchis* for which they proposed the name *Diorchis nyrocae.* The specific name of *nyrocae* was pre-occupied by a member of this genus described by Yamaguti in 1935. Schultz (1940) recognized the existence of these identical names for the two different species and because the *D. nyrocae* of Yamaguti had priority, proposed the new name *D. wigginsi* for the *D. nyrocae* of Long and Wiggins. Schmelz (1941) also noted that these two species had the same name and, apparently unaware that Schultz (1940) had already changed the nomenclature, proposed the name *D. longae* for the *D. nyrocae* of Long and Wiggins (1939). As a result the literature now contains the two names for the species described by Long and Wiggins; however, the name *D. wigginsi,* established by Schultz (1940) has priority by virtue of the first reviser principle and the names *D. nyrocae* Long and Wiggins (1939) and *D. longae* Schmelz (1941) are synonyms.

DIFFERENTIATION: Although such characters as spinose suckers and spinose cirri are not uncommon in the genus *Diorchis,* according to descriptions, only three species, viz., *D. nyrocae* Yamaguti, 1935, *D. spiralis* Szpotanska, 1931, and *D. flavescens* (Krefft, 1871) Johnston, 1912, are provided with both. *D. longiovum* is readily differentiated from *D. nyrocae* and *D. spiralis* on the basis of rostellar hook size and shape as well as size and extent of the cirrus sac. *D. longiovum* is most comparable to *D. flavescens;* however, the latter has a smaller scolex (195 μ), a much shorter rostellum (130 μ) and a considerably shorter cirrus sac (270-350 x 50 μ). The cirrus of *D. flavescens* is finely spinose whereas that of *D. longiovum* is coarsely spinose and the spines occur only at the proximal end. There is no indication that the pocket and margin of the suckers of *D. flavescens* differ in spine sizes as in *D. longiovum.*

Because of frequent loss of spines from suckers and cirri under certain conditions prior to, or during preparation of specimens for study, spinose condition of these organs may not have been observed and consequently not reported in original descriptions. The new species was therefore compared with other members of the genus *Diorchis* having rostellar hooks of similar size and a cirrus sac which extends beyond the median line of the proglottid. The three species comprising this category were found to differ from *D. longiovum* as follows:

D. americana Ransom, 1909 (parasitic in Gruiformes and Galliformes) has a smaller scolex (160 x 250 μ), a less prominent rostellum (50 x 135 μ when extended) and somewhat larger rostellar hooks (65 μ) of a different shape. The cirrus is reported to be unarmed.

D. spinata Mayhew, 1929 (parasitic in Anseriformes) has an ovary which is only slightly lobed, a much smaller cirrus (150 x 20 μ), and smaller rostellar hooks (46-48 μ). The spinose condition of the cirrus illustrated by Mayhew (1929) is similar to that of *D. longiovum,* but sucker armature is not indicated. The eggs are similar in size (69-94 x 12-16 μ) but have a cylindrical shape.

D. formosensis Sugimoto, 1934 (parasitic in Anseriformes) has a much larger scolex (336-392 x 370-420 μ), slightly lobed testes, and a much smaller cirrus sac (233-280 x 21-28 μ). According to the description of this species, only the margin of the sucker is spinose. Cirrus spination is not indicated. The rostellar hooks are only slightly larger (60 μ) but are of a distinctly different shape.

The differential sucker spination found in *D. longiovum* and the characteristic structure of the eggs are distinguishing features which facilitate ready recognition of this species.

AN UNUSUAL OCCURRENCE OF EGGS IN THE VENTRAL EXCRETORY CANAL IN A PARATYPE SPECIMEN OF *D. longiovum*: During routine morphological study of whole-mount specimens of *D. longiovum,* the writer observed rather uniform bodies occurring in an antero-posterior line on the poral side of the majority of the proglottids throughout the strobila of a paratype specimen. These bodies were first noted in the anterior region of the strobila and could not be associated with any genital *Anlagen* characteristic of this species and were thought to be of extra-strobilar origin. Closer examination under oil immersion revealed that these objects seen under lower magnification were the dense constituents comprising the central mass of incompletely developed eggs. These eggs were contained within the lumen of the ventral excretory canal. The spherical egg mass, averaging 30 μ in diameter, was surrounded by a translucent substance, and the whole enclosed by a thin, almost imperceptible membrane. The egg dimensions at this stage of development averaged 32 x 36 μ. In an effort to determine how these immature eggs had escaped the uterus and gained access to the lumen of the ventral excretory canal, the canal was carefully traced throughout the entire strobila. A single early gravid proglottid, 24.5 mm. from the posterior end of the strobila, was partially empty of eggs and proved to be the source of the eggs found in the excretory duct. Critical examination of this proglottid disclosed that an opening existed between the ventral wall of one of the aporal sacculations of the uterus and the poro-ventral wall of the ventral excretory canal about 78 μ in diameter (fig. 5). The smooth appearance of the juncture of the walls of the uterus and excretory duct surrounding the opening indicated the development of abnormal tissue adhesion of these organs in such a manner that an enclosed passage had been formed between them. The eggs remaining within the uterus were quite abundant at the poral end, gradually becoming less numerous towards the middle, leaving the uterus nearly empty in the vicinity of the opening to the excretory duct. Eggs appeared in the passage between the uterus and excretory canal and were distributed discontinuously anteriorad throughout the canal for a distance of 51.8 mm. (including 380 proglottids) and posteriorad for a distance of 9.1 mm. (including 33 proglottids). The eggs found in the excretory canal were identical

in size, shape and stage of development with those remaining in the partially evacuated uterus.

There were no crushed or distorted eggs, ragged parenchyma or other evidence of rupture due to mechanical damage of the proglottid which exhibited this peculiar phenomenon. In consideration of this, together with the extent of distribution of eggs throughout the excretory canal, it is indicated that this unusual development occurred in the living worm.

If it could be assumed that the eggs were washed out of the uterus and transported by the movement of the fluid within the excretory canal, it would seem, in view of the much greater distance anteriorad that the eggs were found in the excretory canal, that the flow of the contents, at least in the poral canal, is in an anterior direction. This is contrary to the usual concept concerning the direction of flow of fluid in the ventral canals. Wardle and McLeod (1952, p. 24), in their discussion of the excretory system of cestodes, stated that, "On each side, a vessel lying somewhat ventrally in the parenchyma and usually of wide lumen carries a fluid—presumably water—in a direction *away from* the holdfast. This is commonly termed the ventral vessel or ventral canal." It is possible that a differential pressure exists between the contents of the uterus and that of the excretory ducts which may, in part, account for the unequal anterior and posterior distribution of eggs in the excretory canal in this instance. There appeared to be no evidence that the eggs were forced out of the uterus and into the excretory canal due to segmental contraction in this unusual proglottid.

There probably are other factors involved in the mechanics of egg movement and distribution seen in this specimen, although they are not apparent in the preserved state. If it can be considered that the fluid in the excretory canal is primarily responsible for the transport of the eggs in this specimen, it would seem that this condition is strong presumptive evidence to support the theory that the excretory system of a cestode is not exclusively excretory in function, but may serve to maintain a hydrostatic pressure within the worm and to regulate its water balance. The differential and discontinuous distribution of eggs in the excretory canal suggests that the direction of flow of the fluid may be anteriorad as well as posteriorad, depending upon the physiological requirements of the worm in different parts of its strobila at any given time.

This specimen of *D. longiovum,* designated a paratype, has also been deposited in the Helminthological Collection of the U. S. National Museum, slide No. 47861.

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Heterodera leptonepia, n.sp., A Cyst-Forming Nematode Found in Soil with Stored Potatoes

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A new species of the nematode genus *Heterodera* was recently found in a sample of cysts submitted for identification by Mr. P. F. Frink of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. This material had been collected by Inspector C. H. Oatridge at the Oakland, California port of entry. The cysts had been washed from about 3 lbs. of soil with small potatoes, part of approximately 3400 lbs. of potatoes taken on as ship's stores at Callao, Peru, and presumably grown in that country. Mr. Frink was informed that the ship plies the Pacific west coast almost entirely on the nitrate run from Peru and Chile to the United States of America. Of the 15 cysts in this sample, 12 were the well-known golden nematode of potatoes, *Heterodera rostochiensis* Wollenweber, 1923[1] and three were a new species herein described as *Heterodera leptonepia*, n.sp. Two of these cysts contained eggs with larvae, and the strikingly slender appearance of these larvae indicated that they were different from any known species of the genus.

Heterodera leptonepia, n.sp.

CYSTS: Light brown, more or less ovate, about 0.5 by 0.3 mm., with distinct neck; smoothly rounded posteriorly as in *H. rostochiensis* and *H. punctata* Thorne, 1928. Vulvar opening round and much larger than the minute, pore-like anus, as is the case with *H. rostochiensis;* different from *H. punctata* which has anus located at a thin spot of cyst wall so that vulvar and anal openings appear about same size (Franklin 1940). Outer layer of cyst wall with rugose pattern of striae extending from neck to near vulva; immediately around vulva, striae interrupted, forming an irregular pattern as shown in figure 1D. A lower layer of the cyst wall distinctly punctate, with minute dots arranged in closely spaced parallel rows at right angles to axis of cyst; dots irregularly spaced in rows.

LARVAE: Length 520μ to 600μ (average 567μ based on 20 larvae from single cyst). Ratio of length to breadth of larvae at widest part of body (a) 39; ratio of body length to length of oesophagus (β) 2.7; ratio of body length to tail length (γ) 9.1. Larvae much more slender than in other known species of *Heterodera*. Head set off by constriction; no annules seen. Lateral fields with four faint lines or grooves, apparently ending shortly posterior to anus. Phasmid not located. Stylet about 18μ long, slender, with knobs convex anteriorly. Orifice of dorsal oesophageal gland about 12μ, or two-thirds stylet length, posterior to stylet; compared with other known species of *Heterodera*, somewhat farther by measurement and considerably farther in relation to stylet length. Middle bulb of oesophagus ellipsoidal. Dorsal oesophageal gland forming a more or less distinct lobe on dorsal side of body; two subventral glands forming a much elongated lobe on ventral side of intestine. Excretory pore about opposite nerve ring. Anlage of genital organs visible as group of cells at about two-thirds of body length from anterior end. Hyaline posterior portion of tail longer than stylet, a little less than one-third of tail length. Terminus very sharply pointed. Larva shown in figure 1A was liberated from the preserved egg by gentle pressure on the cover glass of the slide on which it was mounted. It did not straighten out as a living

[1]Recently reported from Peru (Bazan de Segura, C. 1952. The Golden nematode in Peru. Pl. Dis. Rptr. 36:253.)

specimen would, but retained, to some extent, the seven flexures it had in the egg. Preserved larvae of *H. rostochiensis* show about four flexures.

EGGS: Average length about 95μ; average width about 45μ. Shell without visible markings.

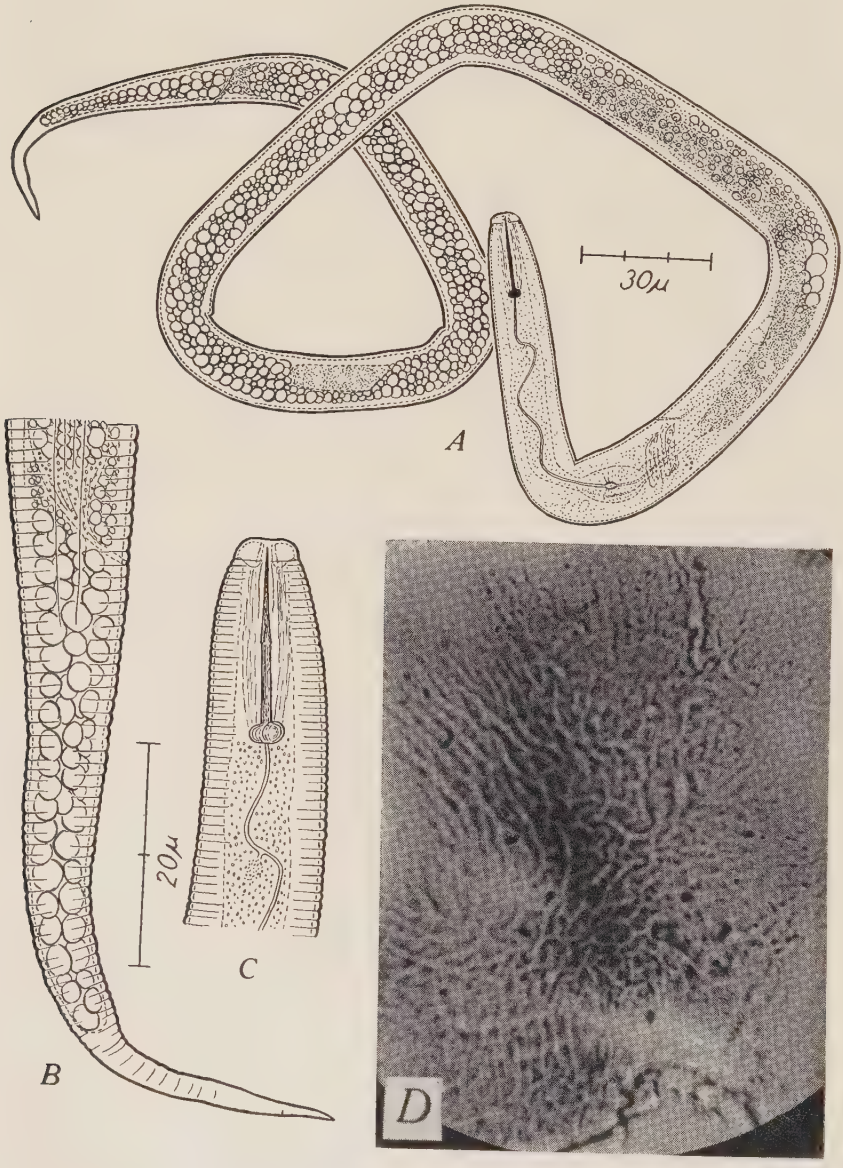

Figure 1. *Heterodera leptonepia*, n.sp. A. Larva from cyst preserved in formalin. B. Tail of larva. C. Head of larva. D. Pattern of cyst, the edge of the vulvar opening at bottom.

DIAGNOSIS: *Heterodera* with cyst rounded posteriorly, therefore without protruding vulva. Anus a minute pore. Larvae very slender; stylet knobs anteriorly convex; orifice of dorsal oesophageal gland about two-thirds stylet length posterior to stylet; hyaline portion of tail less than one-third length of tail. Formula: $a = 39$, $\beta = 2.7$, $\gamma = 9.1$.

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An Unsuccessful Attempt to Protect Mice Against *Schistosoma mansoni* by Transfer Of Immune Rat Serum[1]

M. A. STIREWALT[2] AND A. S. EVANS[3]

Recent emphasis on immunological studies in the schistosomiases has stimulated interest in the possibility of passive immunization of susceptible hosts by the transfer of serum from infected animals. Because of numerous questions along this line recently put to the authors, data are here presented which had been put aside in 1948 on account of their negative conclusions.

During an evaluation of the suitability of the albino rat as a definitive host for *Schistosoma mansoni* (1), a host resistance was noted which was potent enough to destroy the entire parasite burden in most of the rats. Variations in the rate of worm destruction with the degree of infection, together with the fact that the rats were able to effect self-cures even when they had been exposed to as many as 500 carcariae, suggested that a serum antibody factor might be active in the rat. Consideration of the possibility of passive transfer of this factor to mice which are normally susceptible animals quite naturally followed. The work reported here was designed to test this possibility.

MATERIALS AND METHODS

One hundred-seven young albino rats weighing about 125 g each were exposed individually by their partial immersion in infected water—20 the first week, 14 per week for the succeeding three weeks, and 9 per week for the next five weeks—to large numbers of cercariae (7,000-10,000). At the end of eight weeks when the immune response was judged to have reached its peak (2), two rats from the appropriate group were anesthetized with ether and as much blood as possible was withdrawn from the external jugulars and pooled in a conical centrifuge tube. After coagulation was complete, the clot was loosened from the sides of the tube and the serum separated by centrifugation at high speed for 10 minutes. The rats were then autopsied to ascertain the extent of gross manifestations in the liver tissue of response to the infection.

[1]From the Naval Medical Research Institute, Bethesda, Maryland. The opinions contained in this paper are those of the authors. They are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department.

[2]Lieutenant, MSC, U. S. Navy.

[3]HMC, U. S. Navy.

Albino mice were selected as the experimental definitive host for the schistosomes because of their well-adjusted relationship with limited numbers of parasites. The mice employed were of the Carworth Farms-Webster strain which has been maintained since 1937 as an inbred Webster strain by the National Institutes of Health, Bethesda, Maryland. Young animals of both sexes weighing from 14-16 g were used.

These hosts were divided into five main groups as follows:

GROUP I (Serum prophylaxis) (20 mice)—Given 0.3 ml immune rat serum subcutaneously once daily for seven days; exposed to 100 cercariae each on the eighth day; autopsied eight weeks after exposure.

GROUP II (Immediate serum therapy) (20 mice)—Exposed to 100 cercariae each, followed by 0.3 ml immune rat serum given subcutaneously once daily for seven days, including day of exposure; autopsied eight weeks after exposure.

GROUP III (Delayed serum therapy) (20 mice)—Exposed to 100 cerceriae each; four weeks later given 0.3 ml immune rat serum subcutaneously daily during the fifth week of the infection; autopsied eight weeks after exposure.

GROUP IV (Cercarial control) (20 mice)—Exposed to 100 cercariae each; autopsied eight weeks after exposure.

GROUP V (Serum control) (20 mice)—Given 0.3 ml immune rat serum subcutaneously once daily for seven days; autopsied eight weeks after first injection.

Each of the above groups was divided into sub-groups so that an entire unit would not be treated from the same pool of serum nor exposed to cercariae from the same suspension. By way of further advantage, this subdivision made it possible to expose mice from all the groups to cercariae from the same collection, in such a way that the numbers handled at any given exposure or autopsy period were of convenient size. Three-tenths ml of serum per mouse was selected as a maximum safely tolerated daily dosage after preliminary studies to determine sensitivity of the test animals to the heterologous serum. Technics of exposure by partial immersion and autopsy of the mice have been previously described (1).

RESULTS AND DISCUSSION

Of 80 animals exposed, 70 came to autopsy: 12 of group I; 15 of group II; 14 of group III; 15 of group IV; and 14 of group V. While these are admittedly short series, the results were so conclusively negative that further work on passive transfer of immunity was discontinued.

There was one death in group V, the serum control group, but, though it was on the last day of prophylaxis, it was not attributed directly to the serum. All the deaths in the groups of mice which had been exposed to cercariae either before or after attempted immunization were too far removed in time (av 5.8 weeks) from the injections to be attributable either to anaphylaxis or serum sickness.

Analysis of the data in terms of total worm burden per mouse as well as consideration of the sexes separately (table 1) reveals no significant difference between any of the groups. There was no deviation in the experimental animals from the 1:1 ratio of sexes found in the controls; neither was there any difference in the locale of the worms, most of them having been found in the mesenteric vessels. Fecal egg counts were not made, but the worms in the mice were normal in size and appearance and were actively producing

TABLE 1.- Number of *Schistosoma mansoni* recovered from albino mice receiving subcutaneous injections of serum from infected rats, and autopsied eight weeks after exposure to 100 cercariae each, as compared with that from control mice which were infected but untreated.

GROUP I			GROUP II			GROUP III			GROUP IV		
Given .3 ml serum daily for 7 days; exposed on 8th day			Exposed; given 0.3 ml serum daily 1st wk. of infection			Exposed; given 0.3 ml serum daily 5th wk. of infection			Exposed. (Control)		
Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
20	20	40	18	18	36	22	20	42	17	17	34
17	13	30	23	12	35	18	22	40	16	14	30
15	14	29	18	17	35	17	17	34	17	12	29
13	16	29	16	16	32	14	16	30	17	12	29
10	18	28	16	16	32	14	13	27	15	14	29
15	11	26	16	16	32	17	9	26	10	16	26
14	10	24	20	11	31	14	12	26	6	20	26
11	12	23	19	11	30	13	13	26	16	9	25
8	10	18	16	12	28	14	10	24	15	10	25
8	8	16	14	11	25	12	10	22	12	13	25
4	4	8	16	8	24	11	11	22	11	14	25
2	3	5	8	12	20	9	11	20	10	10	20
			9	9	18	8	9	17	10	10	20
			8	7	15	5	5	10	8	11	19
			6	7	13				7	8	15
Average 11.4	11.6	23.0	14.9	12.2	27.1	13.4	12.7	26.1	12.5	12.7	25.1

eggs which were noted in the usual sites. No gross pathological evidence of activity of an immune mechanism was found in any of the mice.

Donor rats, on the other hand, gave evidence of a potent active immunity in many ways: the worms were small; they were restricted to the branches of the portal vein in the liver; eggs were found only in the liver and all of those examined were non-viable; and many dead worms were found walled off in the veins of the liver.

To our knowledge there has been no previous attempt to immunize normal susceptible definitive hosts against the human schistosomes by the transfer of serum from other infected hosts. Such immunization against the nematodes *Nippostrongylus muris* and *Trichinella spiralis* and the cestodes *Cysticercus fasciolaris* and *Taenia pisiformis* has been reported by several investigators whose work has been reviewed by Culbertson (3). To this list, Hearin (4) added *Hymenolepis fraterna*. Other attempts have failed, however, even with these same parasites.

No explanation is attempted here for our failure to transfer immunity from infected rats to mice in these studies. However, the absence of viable schistosome eggs in rats, and therefore metabolites of the miracidia, should be noted.

On the other hand, the presence of a factor or factors inimical *in vitro* to schistosome cercariae has been demonstrated in certain host sera. It is true that these factors have evidenced themselves differently in different hands: Tubangi and Masilungan (5) described "cercaricidal" activity; Papirmeister and Bang (6) found a precipitin; Vogel and Minnig (7) described a "Cercarienhüllenreaktion"; Liu and Bang (8) reported the activity of an agglutinin; the present authors (9) in studies as yet unpublished, in which serum was diluted 1:6 to 1:248 with distilled water suspensions of cercariae, found a lysin in the sera of many different hosts, including the rat, infected with the homologous parasite. Differences in technics may be responsible for this variation in manifestation.

The dose apparently was adequate as compared with the work of others, though the possibility cannot be ignored that a larger dose might have "protected" mice to a greater extent. The total amount of serum administered to the mice was 2.1 ml/20 g mouse. Immunity against *C. fasciolaris* was transferred passively to normal rats from infected rats or rabbits by injection of 1 ml/525 g of rat (10), while Sarles and Taliaferro (11) protected rats against *N. muris* with 4.1 to 6 ml hyperimmune rat serum per 100 g of rat. It may be, of course, that antibodies present in rat serum are destroyed in the mouse, although precedent argues against this (10).

Summary and Conclusions

Seventy albino mice, 41 of which had been given 2.1 ml of immune rat serum subcutaneously in divided doses, immediately before, just after, and 4 weeks after exposure to 100 cericariae each of *Schistosoma mansoni* were autopsied and the adult worms recovered and counted. No significant difference in worm burden was found between any of the groups and the 29 controls, which had received neither serum prophylaxis nor therapy.

From the above results, it is concluded that, at the dosage level employed, the active immunity of albino rats to *S. mansoni* was not established passively in albino mice by the transfer of serum.

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Host Specificity of *Heterakis spumosa* Schneider, 1866 (Nematoda: Heterakidae)[1]

PHILIP E. SMITH[2]

Heterakis spumosa has been reported as a natural infection in *Rattus norvegicus, Rattus rattus,* and *Cricetomys gambianus* (Hall, 1916). Winfield (1933) ran a single experiment to test the infectivity of *H. spumosa* for white mice, and found that they could be infected. In the course of experimental studies on *H. spumosa,* opportunity was afforded to feed embryonated eggs of this parasite to cotton rats, golden hamsters and guinea pigs. These common laboratory animals have never been reported as hosts, either natural or experimental, for this parasite.

COTTON RATS. An initial experiment was set up in which four helminth-free cotton rats, *Sigmodon hispidus hispidus* Say and Ord, were fed a large but undetermined number of infective eggs of *H. spumosa.* After five days one of the cotton rats died, and upon necropsy, *H. spumosa* larvae were recovered from the large intestine. A second cotton rat was killed 14 days after infection, and of larvae recovered, some were in the pre-third mold stage and others had already molted. Numerous larvae were also recovered

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when the third rat was killed, 19 days after infection. In this animal the worms were somewhat retarded in development and there was no indication of the bulge of the forming pre-anal sucker of the male worm. The last rat was examined 47 days after infection, and only mature adults were recovered, the females containing fertile eggs. Eggs were recovered from the rat feces by salt flotation. Eggs obtained by dissecting the female worms were incubated for a suitable period and then were used to infect other rats.

An experiment was set up for the purpose of comparing infections in cotton rats and the hooded laboratory rats. A group of seven cotton rats was matched with a litter of hooded rats born the same day. Both groups were weaned 22 days after birth, and each animal received 300 infective eggs the following day. During the experiment three cotton rats died and corresponding hooded controls were removed. All the remaining animals were killed 41 days after infection. The number of worms recovered from the cotton rats ranged from 0 to 34 with an average of 12.5 worms per rat. In the hooded controls the number of worms recovered ranged from 78 to 110 with an average of 99 worms per rat. The percentage development was 4.1 for cotton rats and 33 for the hooded controls. In salt flotations on feces eggs first appeared on the 31st day after infection in hooded rats and on the 32nd day in cotton rats.

GOLDEN HAMSTERS. A pair of old hamsters was fed an undetermined number of infective eggs, pooled from several cultures. Salt flotations were run on the feces each day from the 28th to the 50th day after infection but no eggs were ever recovered. The animals were then killed and thoroughly examined but no worms were found. To rule out the possibility that age resistance affected the result it was decided to repeat the experiment using young animals of known age with a given dosage of infective eggs. Four hamsters were selected and weaned at 15 days of age, and infected four days later. Controls were four hooded rats weaned and infected at 21 days of age. Each animal was given 600 infective eggs. One hamster and its control were necropsied at intervals from 30 to 67 days after infection, but in no case were worms recovered from the hamsters. On the other hand, normal infections were obtained with the laboratory rat controls.

GUINEA PIGS. Two guinea pigs were given 1800 infective eggs each. One was killed two days later but no larvae were recovered. The second animal died 16 days later, and when it was examined it was also negative for *H. spumosa*. Subsequently, two more guinea pigs only 38 days old were given several hundred infective eggs apiece. One animal was necropsied after 30 days; no worms were recovered from the intestine. The second guinea pig was killed after 48 days and was negative also.

DISCUSSION

In surveys of the parasites of cotton rats (Baylis, 1945; Harkema and Kartman, 1948; Melvin and Chandler, 1950) *H. spumosa* has not been reported as occurring in this host, and to the writer's knowledge, this parasite has not been reported previously from the cotton rat. Experimentally, however, it seems to go through regular development in this host. In the few cotton rats experimentally infected there was a much lower percentage development than in the laboratory rats, but the prepatent period was essentially the same in both hosts. This was similar to results obtained in Winfield's test of infectivity of this parasite for white mice. He found that the average worm burden and the percentage development of the worms was significantly

less than in rats, but that those worms which did develop in mice seemed to grow at a rate similar to that in the rat.

SUMMARY AND CONCLUSIONS

The cotton rat, *Sigmodon hispidus hispidus* Say and Ord is here reported for the first time as an experimental host for *Heterakis spumosa*. The prepatent period is essentially the same for cotton rats as for laboratory hooded rats, but the percentage development is lower in cotton rats. Natural infections apparently have not been found.

Although only a few animals were used, it can be said, tentatively, that both golden hamsters and guinea pigs are refractory to infection with *H. spumosa*.

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The Awl Nematode, *Dolichodorus heterocephalus*, a Devastating Plant Parasite

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Large numbers of the awl nematode, *Dolichodorus heterocephalus* Cobb, 1914, have been found associated with depleted root systems and severe stunting of celery and sweet corn in many fields in the region of Sanford, Florida. Field symptoms of root injury indicated that ectoparasitic nematodes were involved, and in two fields where the awl nematode was numerous no other known plant parasitic nematode was found in sufficient numbers to account for the injury. In one of these, a large field of celery, a reduction in yield of more than 50 per cent was attributed largely to *D. heterocephalus* injury. Soon after seedlings had been set in this field large areas appeared where the plants made little or no growth. Examination of plants from these areas showed that new root growth was almost non-existent. In addition to the Sanford region, the disease has been found near Orlando, Florida, associated with stunting of waterchestnuts growing in hydroponic beds.

The purpose of this paper is to report the results of field observations on the awl nematode and of experiments begun in 1951 to verify conclusions drawn from these observations.

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SYMPTOMS.—The awl nematode feeds from the surface of the roots by injecting its long stylet into root cells, mostly at root tips. Specimens have never been found inside root tissues and only on rare occasions have they been observed attached to the roots. While the stunting of plants is due largely to devitalized root tips, the nematodes are capable of feeding on the surface of succulent roots of considerable size, sometimes even on the hypocotyl. The plant responds to root tip injury by initiating new root buds which also may be attacked before or soon after emerging. The result is a skeleton root system with practically no feeder roots and few, if any, secondary roots. Sometimes one observes considerable decay in the form of dead roots or necrotic lesions but this may be due partly to secondary invaders. The above-ground portions of affected plants show symptoms similar to those caused by any condition that interferes with normal root functions.

The symptoms of injury caused by *D. heterocephalus*, are almost identical to those produced by *Belonolaimus gracilis* Steiner, as described by Christie, Brooks, and Perry (1952) and somewhat similar to those produced by *Trichodorus* sp., as described by Christie and Perry (1951a). Each of these three organisms feeds primarily at root tips, causing a cessation of growth. None of the three is likely to be found within roots, hence, in order to determine which may be injuring plants, one must examine soil taken from the rhizosphere.

THE ORGANISM.—*Dolichodorus heterocephalus* was first described by Cobb (1914), who collected his specimens from Douglas Lake in Michigan and from Silver Springs in Florida. Steiner (1949) reported the occurrence of the nematode in celery fields near Sanford, Florida and referred to it as an ectoparasite. Tarjan (1952) inoculated waterchestnuts with specimens collected from a Sanford celery field and obtained a reduction in both root and total plant growth. Tarjan, Lownsbery, and Hawley (1952) produced stunting, chlorosis, stubby secondary roots, and a reduction in the number of feeder roots by inoculating celery with the awl nematode.

D. heterocephalus is a relatively large and slender nematode with an exceptionally long stylet. It closely resembles *Belonolaimus gracilis* in size, in shape, and in many of its morphological characters. The two can be differentiated easily by the shape of the tail. The female of *B. gracilis* has a blunt, broadly obtuse, rounded tail and the male a long narrow bursa, whereas the female of *D. heterocephalus* has a bluntly pointed tail and the male a short wide bursa.

The awl nematode occurs naturally in wet locations or at least it can live and thrive in such an environment, as evidenced by the specimens collected in hydroponic beds. In tilled fields it is favored by moist conditions such as those found in the sub-irrigated land of the Sanford area. In Florida this nematode has been collected from the vegetable land of the Sanford area, from hydroponic beds near Orlando, from a strawberry plant bed in Sumter County, and from the water of Silver Springs; in Georgia from a corn field near Tifton. It has been demonstrated that the awl nematode will feed on and injure the roots of beans (*Phaseolus vulgaris* L.), corn (*Zea mays* L.), celery (*Apium graveolens* var. *dulce* (Mill.) Pers.), tomato (*Lycopersicon esculentum* Mill.), and waterchestnut (*Eleocharis dulcis* (Burn. F.) Henschel) but undoubtedly this list is far from complete.

EXPERIMENTAL RESULTS

The nematodes used as inoculum for these experiments were obtained

from field soil and were removed by a combination of sieving and the Baermann technic as described by Christie and Perry (1951b). The soil in which the experimental plants were grown was fumigated in an air tight box with chloropicrin applied at the rate of 5 cc per cubic foot. Two methods were used for inoculating the plants.

In the first method, used for the more critical tests conducted in 6-inch pots, nematodes were removed one by one with a bamboo pick and transferred to clear water. This water suspension was then pipetted to soil surrounding the seeds or the roots of the seedlings. In the second method, used for experiments conducted in flats, all the nematodes, as drawn from the funnels, were used as inoculum. With this mass transfer procedure, various kinds of nematodes are included but, as a source of material, soil was collected in which a high percentage of the nematodes were *D. heterocephalus* and that contained no, or very few, other plant parasitic species. Because the awl nematode is large, a coarse sieve (90 meshes to the inch) was used which eliminated many of the small forms.

In each of the experiments a series of test plants was grown. As soon as one set of plants was removed for examination the pots or flats were replanted immediately without the addition of any inoculum. For all the experiments in pots where beans or corn was used as test plants three seeds were planted in each pot. When more than one seed germinated, the stand, in most cases, was thinned to one plant per pot. Where tomatoes, celery or peppers were tested transplants were used, one to a pot.

EXPERIMENT 1. One of a pair of 6-inch pots was inoculated with 1000 picked specimens of the awl nematode while the other pot served as a control. Sweet corn seeds germinated in both pots and the plant in the infested pot made a fair growth, but its roots showed typical and moderately severe symptoms of nematode injury. Snap beans followed corn in this experiment and the plant in the inoculated pot was severely stunted and its roots severely injured. A total of 1,758 specimens of the awl nematode was obtained when the root system of this plant, with no adhering soil, was placed in a Baermann funnel. Tomato plants followed the bean plants and the root system of the one in the inoculated pot was almost completely destroyed. The 1,758 specimens removed from the bean plant were used to inoculate the soil in a greenhouse flat. Germination of sweet corn seeds planted in this flat was not prevented but definitely delayed.

EXPERIMENT 2. Three snap bean seeds were planted in each of two pots, the soil in one of the pots having been inoculated with 1,000 picked specimens of the awl nematode, mostly females. All the seeds in the inoculated pot failed to germinate while all the seeds in the control pot grew. Thirteen days after planting, a microscopical examination of the seed from the inoculated pot revealed the presence of the nematodes both inside and outside the seed coat. These seeds were swollen but showed no other evidence of germination and they were not decayed. Apparently the nematodes had fed on the embryo and stopped its growth.

Three bean seeds were again planted in each pot and, as before, all three seeds of the control germinated. In the inoculated pot one seedling finally emerged but it remained extremely stunted (Fig. 1). The two bean seeds that failed to germinate were partly decayed but apparently growth of the embryo had been stopped.

Sweet corn seeds, planted in each pot after the second bean plants had been removed, germinated normally but the seedling in the inoculated pot

was extremely stunted (Fig. 2, B). After 5 weeks the corn plants were removed and the roots of the affected one, with no adhering soil, were placed in a Baermann funnel. About 15 hours later 2,303 specimens of the awl nematode were removed from the funnel.

Peppers followed sweet corn in this experiment. The plant grown in the inoculated pot was stunted and its roots were injured but the injury was much less severe than that inflicted on the roots of such plants as tomato.

The 2,303 specimens mentioned above, when used in another inoculation experiment conducted in crocks, completely inhibited the germination of three sweet corn seeds.

Experiment 3. One celery seedling was transplanted into each of two pots and 600 picked specimens of the awl nematode were placed around the roots of one of the seedlings at the time of transplanting. Stunting of this inoculated plant became apparent in a very short time. When examined 8 weeks after transplanting the roots of the affected plant showed symptoms similar to those seen on celery plants grown in infested commercial fields (Fig. 1).

Tomato plants followed the celery plants in this experiment and the one in the inoculated pot made no growth. The nematodes not only prevented this plant from forming new roots but they destroyed the roots it already had when transplanted (Fig. 2, A).

The awl nematodes were removed from the surface of the roots of the affected celery plants mentioned above and about 500 were used to inoculate one of a pair of pots in which bean seeds were planted. The bean plant in the inoculated pot was injured but not severely. Sweet corn seed, when planted in this inoculated pot after the bean plant was removed failed to grow. The nematodes were observed in a feeding position on the surface of the radicle and growth of both the radicle and the plumule had stopped before the seedlings emerged.

Experiment 4. Two greenhouse flats, each containing 6 celery seedlings, were inoculated with the awl nematode by the mass transfer method. Two other flats, treated in the same manner but not inoculated, served as controls. The celery plants grown in the uninoculated flats produced normal root and top growth as compared with severe stunting of those grown in the inoculated flats. Root symptoms of these affected plants were similar to those observed in commercial celery fields infested with large numbers of the awl nematode. Sweet corn, which followed the celery in this experiment, was severely stunted in the inoculated flats but made a normal growth in the controls.

Comments and Conclusions

Although present records indicate that the awl nematode is not as common or widespread in Florida as the sting nematode, where it occurs it can be equally destructive to crops. Severe root injury and correspondingly severe stunting of growth has been produced experimentally on celery, corn, bean, and tomato plants and moderate injury on pepper plants. This injury on some of these experimental plants was the most severe nematode damage that the writer has had occasion to observe. Although primarily a root-tip parasite, this nematode has the ability, perhaps to even a greater extent than the sting nematode, to feed along the sides of roots and even at the base of the hypocotyl.

The awl nematode, believed to be normally an inhabitant of wet loca-

tions, is capable of living and thriving in soil saturated with water. Flooding, a procedure sometimes practiced in the Sanford area, would therefore have no value as a control measure. The awl nematode may prevent seed from germinating by penetrating the seed coat to feed on and devitalize the embryo.

FIG. 1. Two pairs of pots in which experiments were conducted with beans (Expt. 2, pair on right) and celery (Expt. 3, pair on left). Control plant on right of inoculated plant in each case. Bean plant inoculated with 1,000, and celery plant with 600, picked specimens of *Dolichodorus heterocephalus*. Roots of plants shown below. Photographs taken at end of 33 days except roots of celery plants which were photographed at end of 61 days.

Failure of seed to germinate causes much trouble and loss to farmers in the South. Also, it is of serious concern to seedsmen who are continually being blamed, and sometimes sued, for supplying poor seed. Trouble of this kind seems to be especially serious in regions where ectoparasitic nematodes are known to be numerous. These nematodes may prevent the germination of seed in two ways. (a) By penetrating the seed coat they may feed on and devitalize the embryo before growth takes place. Only the awl nematode has been definitely demonstrated to have this ability although it seems probable that the sting nematode can do the same. (b) After the seed has germinated these nematodes may feed at the growing tip of the shoot to kill or devitalize it, resulting in a kind of preemergence "damping off." Apparently any of the root tip parasites such as the awl, sting, or stubby-root nematodes may prevent emergence in this way.

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Fig. 2. A—Tomato plants that followed celery plants shown in figure 1 (Expt. 3). B—Corn plants that followed bean plants shown in figure 1 (Expt. 2). Photographs taken about 1 month after pots were replanted.

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The domestic cat a new host for *Capillaria plica* in North America

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Although there are several reports from North America of the occurrence of *Capillaria plica* (Rudolphi, 1819) Travassos, 1915, in the urinary bladder of dogs (*Canis familiaris*) and foxes (*Vulpes* spp.), there appears to be no record of this species from the domestic cat (*Felis catus domesticus*) on this continent. *C. plica* has been reported from this host in Germany (Wagner, 1936), Puerto Rico (Price, *in* Van Volkenburg, 1937, 1939), and Yugoslavia (Erlich, 1938), but the species most frequently reported from the bladder of the cat throughout the world is *C. felis-cati* (Diesing, 1851) Travassos, 1915.

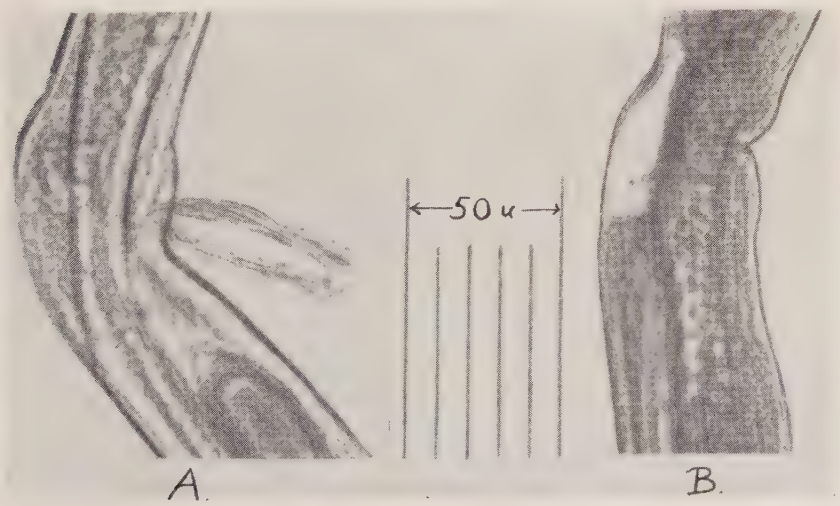

FIG. 1. The vulvae of capillarids occurring in the urinary bladder of cats and dogs. A. *Capillaria plica;* and B. *Capillaria felis-cati.* Photographs were taken at the same magnification.

Lewis (1927 a) reported *Capillaria linearis* from the urinary bladder of cats in Wales, but the writers agree with Read (1949 a) who considered it unlikely that these specimens from the bladder are co-specific with *Capillaria linearis* (Leidy, 1856) Travassos, 1915, from the intestine. Rather, Lewis' specimens, as well as the *Capillaria* spp. reported from the bladder of cats by Lewis (1927 b) and Chen (1934), are probably referable to *C. felis-cati* or *C. plica*.

As noted in a previous article (Enzie, 1951), *C. felis-cati* is encountered occasionally in domestic cats obtained in the vicinity of Beltsville, Maryland. On December 8, 1950, several specimens of *C. plica* were recovered from a cat obtained in this area. There were no records in the Helminthological Collection of the U. S. National Museum of this species from domestic cats, and an inquiry to each of the North American Veterinary Colleges disclosed no records of *C. plica* from this host at any of these institutions. Presumably, therefore, this is the first report of *C. plica* from domestic cats in North America.

C. plica is readily distinguished from *C. felis-cati* by the tubular appendage on the vulva of the former, Fig. 1. In this connection it may be noted that Read (1949 b), in a diagnostic key to the species of *Capillaria* in North American mammals, characterized the vulvar appendage of *C. plica* as campanuliform or bell-shaped. Similarly, Teixeira de Freitas and Lent (1936), in a monograph on *Capillaria* in mammals, stated that the vulva of *C. plica* is campanuliform (p. 89) although their drawing of the vulvar region of this species (Plate 1, Fig. 5) clearly shows a tubular appendage. Other differential characters as well as photomicrographs of the eggs of *C. plica* and *C. felis-cati* are given in an earlier report (loc. cit.).

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The use of lead arsenate mixed with Phenothiazine for the removal of tapeworms from sheep and goats

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Apparently Harwood and Guthrie (1940) or Mohler (1939) first reported the use of lead arsenate as a taeniacide, although various other arsenicals have been used in the past as anthelmintics. These workers found that the chemical was very effective at doses of 0.3 gram per fowl, but toxic to a small number of the chickens. Therefore, it was not recommended for use. Other investigators subsequently applied this chemical successfully to the removal of cestodes from ruminants.

Foster and Habermann (1948) have reviewed carefully the literature describing the use of lead arsenate for the removal of tapeworms from ruminants. Therefore, only the more recent literature is mentioned here. Those writers state that removal of tapeworms has resulted in decided improvements in the health of infected animals, and that preliminary experiments are encouraging in this respect. However, they warn that our knowledge of the toxicity is incomplete, particularly in regard to pregnant animals. Allen and Jongeling (1948) found that one gram of lead arsenate was effective for the removal of *Moniezia* from lambs. However, the drug caused a mild enteritis, inappetence, listlessness and one lamb died possibly from the effects of the treatment. Analysis of the tissues revealed up to 3 parts per million of arsenic and 1.8 parts per million of lead.

Morgan, Pope and Sorensen (1950) employed 30 lambs to test the efficacy of lead arsenate. Ten served as untreated controls, ten animals were given 0.5 gram each and 10 one gram of lead arsenate each. The one-gram dose removed 100 per cent of the tapeworms, while the 0.5 gram dose removed only 65 per cent. No gross lesions attributable to lead arsenate could be observed.

Link, Levine, Danks and Woelffer (1950) reported on the treatment of an extensive outbreak of *Moniezia expansa* infection. Nineteen calves out of 79 died and 42 were emaciated, lacked vigor, and grew slowly. Treatment with 0.5 to 1.5 grams of lead arsenate removed the tapeworms and permitted the calves to make an uneventful recovery.

Data at present available indicate that lead arsenate is very effective for the removal of tapeworms from sheep, goats and calves. There remains the question of safety. Probably the recent opinion of Foster and Habermann (1948) summarizes the present position on this question adequately. They consider that the drug can be used safely and effectively, although they point out that our knowledge of its toxicity is as yet incomplete, particularly in regard to pregnant animals. Therefore, the investigations reported at this time are chiefly concerned with the toxicity of a phenothiazine-lead arsenate drench.

EXPERIMENTAL RESULTS

Sheep number 505 (weight not recorded) was given one gram of lead arsenate on February 20, 1946, and confined to a small clean pen. It was given access to water but only oats for feed. Screening of the feces revealed a few segments of *Moniezia expansa*. At necropsy on February 22, 1946, one adult *Thysanosoma actinoides* was found. Apparently all *Moniezia* were removed, but *Thysanosoma* was not affected. No symptoms or gross pathology were observed.

Goat number 506, 68 pounds, was dosed March 10, 1947, with 1 gram of lead arsenate and 25 grams of wettable phenothiazine. In the next three days, 8 *Oesophagostomum venulosum* and 4 *Skrjabinema ovis* were eliminated. No helminths were found at necropsy on March 14. Throughout the experiment the goat showed no evidences of intoxication and no lesions were observed at necropsy.

Sheep number 507, 92 pounds, was dosed with one gram of lead arsenate suspended in water with 25 grams of wettable phenothiazine on March 18, 1947. By means of a sampling technique we estimated that 3,916 *Trichostrongylus* were found in the feces. In addition to numerous tapeworm fragments, 5 *Oesophagostomum columbianum* and one *Trichuris* were found in the feces. Samples taken from the small intestine at necropsy on March 22 indicated that 2,817 *Trichostrongylus* resisted the medication. One *Bunostomum trigonocephalum* was also found at necropsy. This animal was in an advanced state of pregnancy, but no symptoms of intoxication were noted, and no lesions attributable to the medication were found.

Goat number 2383, 53 pounds, was given 5 grams of lead arsenate on April 3, 1947. Subsequently it showed muscular tremors and other colicky symptoms. By April 8 tremors had ceased and the goat seemed to be recovering, but it developed a stiff gait again on April 9. By April 15 it was conspicuously off feed and weighed only 43 pounds. The animal died April 17, two weeks after dosing. At necropsy focal necrosis of the liver, hemorrhagic ulcers in the colon, and general though mild congestion of the intestine were observed. There was also a pronounced terminal pneumonia.

Goat number 2384, 35 pounds, was dosed on July 26, 1947, with 25 grams of phenothiazine and one gram of lead arsenate. The goat was sacrificed July 28. Liver, kidney and lean meat were analyzed by Dr. Noel M. Ferguson, who reported no lead or arsenic in any of the specimens.

On August 23, 1947, goat number 4A was given 25 grams of phenothiazine and 5 grams of lead arsenate. This animal was killed August 25, and Dr. Noel M. Ferguson reported a trace of lead and of arsenic in the liver, but kidneys and muscle tissues were free of the metals. A control animal, from the same herd, showed no lead or arsenic in the tissues.

Goat number 5A, 50 pounds, was dosed with 25 grams of phenothiazine and 5 grams of lead arsenate on August 30, 1947. It died between September 2 and 3. Tissues were sent to Dr. Noel M. Ferguson, who reported "a little lead and some arsenic in the tissues." However, he likewise reported that tissues from another goat, which had not been dosed, showed a "possible trace of arsenic."

Goat number 5772A, 60 pounds, was dosed with 25 grams of phenothiazine and one gram of lead arsenate on September 24, 1947. The treatment was repeated at irregular intervals of 2 to 10 days until by November 3 a total of 14 doses had been administered. The animal exhibited no symptoms of intoxication, and on November 4 a dose containing 375 grams of phenothiazine and 15 grams of lead arsenate was administered. The goat was killed November 4 and tissues sent to Dr. Noel M. Ferguson, who reported a trace of lead and of arsenic in both the liver and the kidney. An undosed control from the same herd showed neither lead nor arsenic in the tissues.

Goat number 7A, 115 pounds, was given 14 pellets, each of which contained 12.5 grams of phenothiazine and 0.5 gram of lead arsenate on August 1, 1951. As expected, the dose proved toxic and the goat died August 5.

On August 30, 1951, we dosed all animals in our experimental goat herd with a prepared phenothiazine-lead arsenate drench. Details are given below.

Goat 8A, a male weighing 90 pounds, was given 14 ounces of the suspension. He died September 4, and at necropsy showed a large quantity of fluid in the body cavity, focal necrosis of the liver, generalized edema of the small intestine and moderate congestion of the duodenum.

Goat number 9A, a male weighing 76.5 pounds, was given 10 ounces of the suspension. He showed a severe diarrhea September 5, and died September 6. Findings at necropsy were very similar to those reported for 8A.

Goat number 10A, a male kid weighing 47.5 pounds, was given two ounces of the suspension. This kid was weak and in a very debilitated condition, probably from gastro-intestinal helminthiasis, at the time of dosing. Two days after dosing he seemed improved, but on the third day he showed pronounced colicky symptoms. On September 6 this goat weighed 30 pounds. He died the next day, presumably of combined arsenic poisoning and gastro-intestinal helminthiasis.

Goat number 10B was a female kid, weighing 30 pounds on Auugst 30 when she was dosed with two ounces of the phenothiazine-lead arsenate mixture. She was in fair condition and showed no ill effects. On September 6, one week after dosing, she weighed 32 pounds.

The remaining ten animals in the herd were not numbered. They were each given two fluid ounces of the drench. They were of various ages; two were young kids, and several showed symptoms typical of parasitic gastro-enteritis. On September 3, one female dropped a normal kid and on September 5, another also produced a normal healthy kid. No animal in this herd showed any symptoms of intoxication, and the general condition of the herd improved rapidly after treatment.

DISCUSSION AND SUMMARY

It has been assumed frequently that a mixture of phenothiazine and lead arsenate is as effective as each drug administered independently. The limited tests described herein seem to support that assumption.

Unfortunately the toxicity of the mixture, and of lead arsenate used alone are not well established. Our tests indicate that there is very little difference between the mixture and the pure metal salt. Both are equally dangerous, since 5 to 7 grams of lead arsenate per dose killed all of the animals to which that quantity was given.

At the prescribed dosage level, however, the drug seems safe for all but extremely debilitated animals. Even sheep and goats in advanced stages of pregnancy showed no ill effects.

One animal was dosed repeatedly with this mixture. It lost a little weight but showed no other symptoms. Dr. Noel Ferguson insisted that the amount of lead and arsenic in the tissues from several test animals was well within tolerances suggested by the Food and Drug Administration, but did not give quantitative estimates.

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Studies on the Variability of Anal Plate Patterns in Pure Lines of *Meloidogyne Spp.* The Root-Knot Nematode

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The taxonomy of the root knot nematodes was reviewed by Chitwood (1949), who based his separation of species of these plant parasites on differences in host range and in the morphology of the worms. This group of organisms was chosen for a long range study of the heredity of a parasite. They offer a complex morphological character (the anal plate pattern), are obligate parasites, and the host-parasite relations have been described for a number of combinations. Descriptions of the life cycle, host-parasite relations, and host range may be found in the papers of Christie. Males occur less frequently than females and it is assumed that occasional bisexual reproduction takes place. Nothing is known of the chromosome behavior in this genus. Among the advantages of *Meloidogyne* spp. as a suitable form for the investigation of the heredity of a parasite is the wide array of host-parasite relations which exist. There are various associations of plant and worm ranging from those which yield high numbers of parasite eggs in a short time, to those in which few or no eggs are produced by the parasite. The degree of galling of the plant seems to be independent of the well-being of the worm. Some roots are entered freely by the larvae, while others apparently are not at all attractive (Sasser, 1952). Another advantage of this combination of nematode and plant is that many host plants reproduce vegetatively. It is therefore possible to hold the host's heredity relatively constant over long periods. This is much more difficult in animal host-parasite associations.

METHODS

Following the lead of Tyler (1933) infections were made by placing single larvae on marked roots of host plants. After 30 days the plants were examined for the presence of a gall and egg mass. Roots were marked with black thread. The larvae were handled in water with a glass loop, under a dissecting microscope, at 30x magnification. The plants were grown in a greenhouse, using vermiculite and nutrient solution. The parent female for any batch of larvae was preserved in formalin and each egg mass was permitted to hatch separately in water in a micro culture slide.

In this fashion over 20 populations of worms have been produced, each tracing its origin in a single larva. One line has been carried continuously by single larva passage and it has just passed its 5th generation. The success of the method is shown in Table 1 which presents a summary of the entire experience with single larva infections arranged according to host plant and species of worm. The combination of *M. incognita* var. *acrita* with All-gold sweet potato is by far the most successful. The line which is now in its 5th generation is an *acrita* which has been transferred on this plant exclusively.

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The opinions or assertions contained therein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

TABLE 1

Infectivity of *Meloidogyne* spp. in single larva exposures

Worm species	Plant Host: Marglobe tomato Number	% positive	Allgold sweetpotato Number	% positive	Coleus Number	% positive
acrita	201	1.4	225	19.1	16	0
hapla	101	1	49	0	16	0
arenaria	84	2.4	69	1.4	36	5.5
Total	386	1.5%	343	12.8%	68	2.9%

Comparisons were made of the variations in anal plate characters among the offspring from single larvae with those among control or "wild" populations. The patterns consist of a series of cuticular rings surrounding the anus and vulva (Fig. 5). These are the annulations that are present in the cuticle of most nematodes. In *Meloidogyne* they serve as the chief taxonomic character. Families of worms were collected as first generation offspring from single larva infections. The single larva families are: *acrita*-17, grown on a single host plant (Marglobe tomato) and preserved on the 55th day after infection; *acrita*-13, grown on 5 host plants of three types (Henderson Bush bean, Coleus, Marglobe tomato) and preserved at various ages, ranging from 28 to 38 days after infection; *arenaria*-19, grown on a single host plant (Marglobe tomato) and preserved at 52 days after infection.

The control populations consist of worms of mixed ancestry, taken from the stocks maintained in the greenhouse. These were propagated from infected roots supplied by A. L. Taylor, of the Division of Nematology, Bureau of Plant Industry, Beltsville, Md. A number of egg masses taken from several pots of the species used were permitted to hatch in a micro culture slide. A plant was then exposed to large numbers of larvae on day 1 and preserved on day 31 so that the worms within the control populations were all of the same age. One control consists of worms of mixed ancestry and of unknown age. The designations of the control or "wild" populations are as follows: *acrita* stock 6—grown on a single host plant (Marglobe tomato) and fixed at 31 days. *acrita* wild undated—grown on several host plants (Marglobe tomato) and fixed at various ages. *arenaria* stock 10—grown on a single host plant (Marglobe tomato) and fixed at 31 days.

Photographs of the patterns were made under oil immersion (40x Fluorite objective) and enlarged in the prints to 1400x magnification. Tracings of the patterns were made from the prints to simplify the classification of pattern types.

RESULTS

I. The first series of tracings were made in the following manner. The prints were oriented with the vulva below the anus. An axis was constructed passing from the center of the vulva through the center of the arch of the pattern above the anus. The line for tracing crossed the axis at a standard distance of 8.1 cm. above the vulva. All the tracings for a given population were superimposed to give a composite figure. Figure 1 is a sample of this type of figure to illustrate the method. There is no measurable difference in variability between the patterns of the single larva progeny of arenaria and of the control population. A tendency for the patterns of *arenaria*-19 to parallel each other may be detected in this figure.

The area of each tracing was measured with a planimeter to compare the variations. Results are presented in Table 2.

Again this system of measurements does not show any difference between the closely related worms and those of mixed ancestry. The variability of population *acrita* wild undated is much greater than that of the other *acrita* populations. These specimens were collected as adults of unknown age and unknown ancestry. Furthermore they had been grown in several pots on separate plants of Marglobe tomato. It seems likely, therefore, that age influences the variability of the anal plate patterns.

II. Measurements of certain landmarks on each specimen were made with ocular micrometer at 900x magnification. These landmarks are the

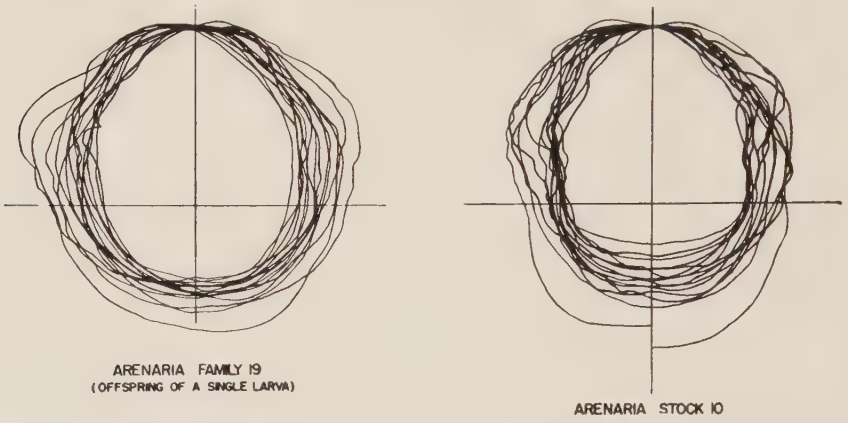

FIG. 1. Tracings from the anal plate patterns of the F-1 offspring from a single larva of *Meloidogyne arenaria* and of a mixed population of this species.

TABLE 2

Variability of areas of anal plate patterns in populations of *Meloidogyne* spp.

Population	Area in Planimeter Units	Coefficient of Variability	Number of Specimens
acrita 17	1.86 ± .083*	17%	15
acrita stock 6	.78 ± .035	17%	16
acrita wild undated	1.33 ± .117	36%	17
arenaria 19*	.99 ± .044	17%	15
arenaria stock 10	.69 ± .031	18%	15

*Standard error of the mean

width of the vulva (A) and the distance between the anus and vulva (B). The distance was determined along a line from the anus, forming a 90° angle to the vulva. Results of these measurements are recorded in Table 3 which includes all the specimens in the populations studied. Measurements are in ocular micrometer units.

Again this system of measurement fails to reveal any consistent pattern of difference in variability between the closely related populations of worms and the populations of mixed ancestry.

TABLE 3

Variability of landmarks on the anal plate patterns of populations of *Meloidogyne* spp.

Population	A = width of vulva		B = distance from anus to vulva		B/A		Number of Specimens
	Mean*	C.V.**	Mean	C.V.	Mean	C.V.	
acrita 17	4.84 ± .11	11%	3.53 ± .09	11%	.74 ± .02	11%	21
acrita 13	2.41 ± .06	13%	1.88 ± .06	17%	.80 ± .03	24%	31
acrita stock 6	2.3 ± .05	8%	1.85 ± .04	9%	.81 ± .02	8%	16
acrita wild undated	4.1 ± .10	12%	3.7 ± .19	24%	.90 ± .04	18%	23
arenaria 19	3.0 ± .09	12%	2.0 ± .06	12%	.68 ± .02	10%	17
arenaria stock 10	2.4 ± .05	8%	1.7 ±.05	11%	.71 ± .02	14%	17

*Mean ± standard error of the mean.
**C.V. = coefficient of variability.

III. Since it seemed that there was actually such a difference when the photographs of each group were compared, a second series of tracings was made. These tracings were chosen with the idea that the important differences in the pattern were closer to the anus than the previous tracings. This second set was made by drawing an axis through the anus from the center of the vulva, and extending this line above the anus to a distance equal to the width of the vulva. The end of this line was used as a starting point for the tracing. Part of this series of patterns is shown in Figures 2, 3 and 4. The patterns from *acrita* stock—6 appear to be more variable than those of *acrita* —17 (single-larva family) on a single host plant, and *acrita*—13 (single larva family on five host plants).

In these figures, the tracing are grouped according to similarity of shape. Analysis of the tracings was made in the following manner. The width of the tracing at the level of the anus was taken for reference. The amount of narrowing above the anus was recorded by measuring the width midway along the axis between the anus and the line of the tracing. The amount of widening below the anus was recorded by measuring the width midway between the anus and the vulva. Each of these measurements was subtracted from the measurement at the level of the anus, and the average of the two was taken as the *pattern index* for a given specimen. Thus if the patterns differ widely in shape the variability of this statistic is high.

Table 4 represents the results of this analysis for five populations. The variability of the wild type *acrita* is twice that of the single larva offspring. In *arenaria, however,* both populations are highly variable.

IV. As a final evidence of the inheritance of the character under study, the results of another series are presented. In the original series of wild type *acrita,* several specimens were noted which differed markedly from the usual type. A search of 10 specimens from each of the six pots of stock plants showed that the odd type occurred among the worms of only one pot, labeled E. The egg mass from an individual of type E was used to infect a plant. The code number for this series is E-3. The first generation yielded only two worms, both with pattern E. The second and third generations consisted of six specimens each.

TABLE 4.—Variability of anal plate pattern shape in populations of *Meloidogyne* spp.

Populations	Mean Pattern Index	Coefficient of Variability	Number of Specimens
acrita—17	9.49 ± .46*	20%	17
acrita—13	9.42 ± .36	19%	25
acrita stock—6	7.13 ± .72	41%	16
arenaria—19	6.03 ± .87	40%	17
arenaria stock—10	5.75 ± .47	33%	16

*Standard error of the mean.

TABLE 5

Counts of lines entering the central area of the anal plate pattern in populations of *Meloidogyne acrita*

Population	Lines at the right	Lines at the left	Lines at the center	Total	Number of specimens
acrita E-3 (parent + 3 generations of offspring)	53	45	13	111	15
acrita stock 6	13	11	2	26	17
acrita—17	4	8	0	12	19

In the more common *acrita* pattern, there are relatively few transverse lines entering the center of the pattern in the area between the anus and the vulva. Type E, however, exhibits many such lines and an attempt was made to express this quantitatively. Figure 5 is a photograph of one of the original specimens first noted in the study of the *acrita* wild undated population. Counts were made of the lines which enter the central area as follows. Most specimens of *acrita* have a line running close to the anus, roughly parallel to the vulva. With a grid type of ocular micrometer, using a phase microscope, lines were located running from each end of the vulva to the ends of the line near the anus. This yielded a figure of trapezoidal shape. Counts were then made of the lines intersecting this figure at the right and at the left. Counts were also made of the lines which intersected the axis from the anus to the center of the vulva. The results of these counts are presented in table 5 in which comparisons are made between the three generation of *acrita* E-3, *acrita* stock 6, and *acrita* 17.

This type of pattern seems to be inherited. It was first noted as an unusual pattern in stock *M. acrita*, but was found with high frequency among the two generations of offspring from a worm showing the pattern.

Discussion

On the whole this study suggests that the general shape and, perhaps, some of the details of the anal plate patterns are under the control of heredity. Population *acrita*—17, derived from a single larva, consisted of worms with a broad vulva and relatively short anus-vulva distance. The center of the area is free of lines. Population *acrita* E-3, on the other hand, has patterns

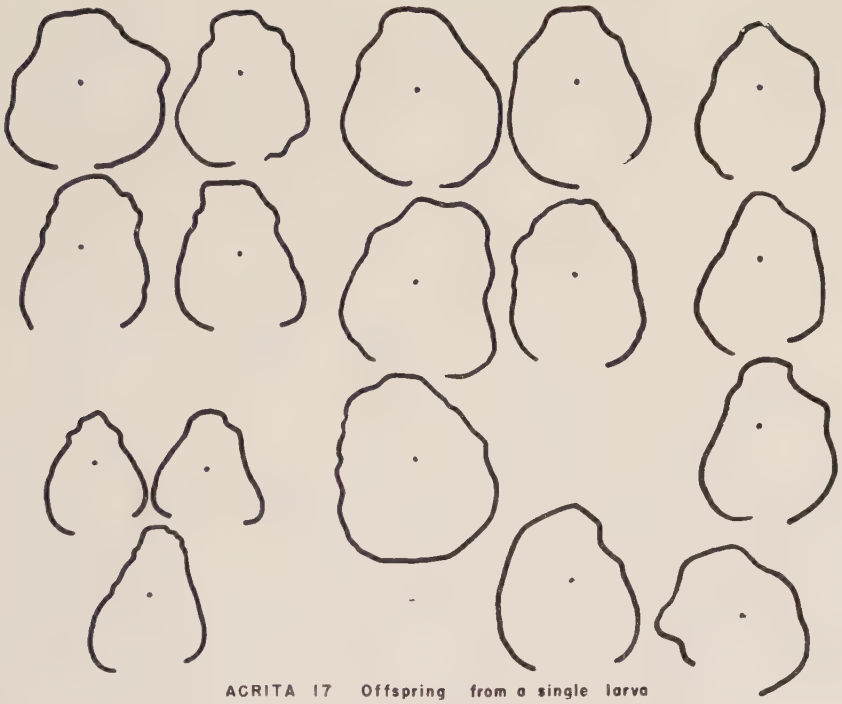

FIG. 2. Tracings of the anal plate patterns of the F-1 offspring from a single larva of *Meloidogyne incognita* var. *acrita,* all of the same age and grown on one host plant.

with a narrow vulva, a relatively distant anus, and with many lines which reach the central field. Population *acrita*—13 is somewhat more elongated than *acrita*—17, and has few lines between the anus and vulva. In this limited series of offspring from single larvae (3 populations, totalling 69 specimens), and in the course of the entire study, not a single case has been observed in which a parent of one species produced offspring which could be classified as belonging to another species. This confirms the taxonomic work of Chitwood (1949) but leaves the observations of Allen (1952) unexplained. It is planned to measure the infectivity, host range, and other physiological characteristics of the various lines to determine the degree to which they differ in these qualities.

SUMMARY AND CONCLUSIONS

1. Six populations of the root-knot nematode were studied to determine the degree of variability in anal plate patterns among the offspring of a single larva.

2. These patterns are less variable in single larva families of *M. acrita* than in populations of mixed ancestry; in *M. arenaria* no differences could be demonstrated.

3. A particular pattern which occurs with low frequency in stock *M. acrita* was found with high frequency among the offspring of a worm showing the unusual pattern.

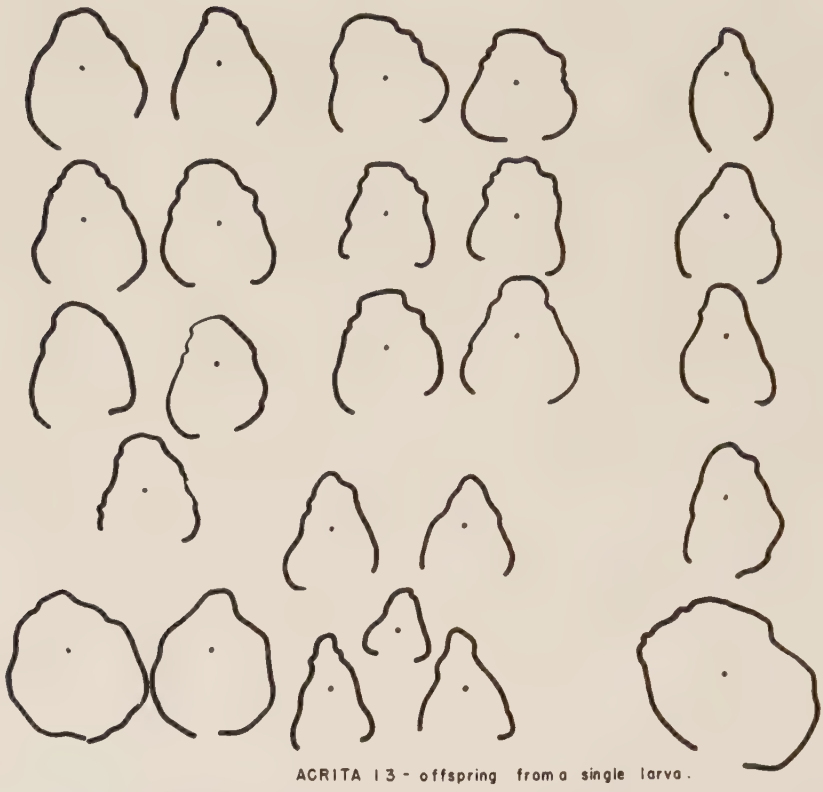

FIG. 3. Tracings of the anal plate patterns of the F-1 offspring from a single larva of *Meloidogyne incognita* var. *acrita,* grown on 5 host plants, and with a 10 day range of age.

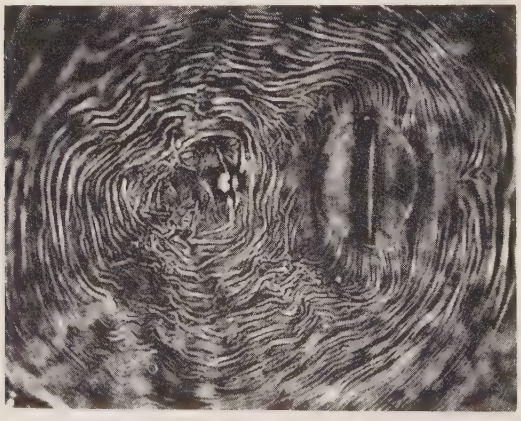

FIG. 5. *Acrita* type E.

FIG. 4. Tracings of the anal plate patterns of *Meloidogyne incognita* var. *acrita*, of mixed ancestry, all of the same age and grown on one host plant.

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Studies on the Miracidium of the Genus *Trichobilharzia* With Special Reference to the Germinal Cells*

D. J. Ameel, Anne Van der Woude, and W. W. Cort

In connection with investigations on the germinal development in the schistosome group we had some opportunity to study the miracidia of three species of the genus *Trichobilharzia*, viz., *T. elvae* (Miller, 1923)[1], *T. physellae* (Talbot, 1936), and *T. stagnicolae* (Talbot 1936). The observations were made on miracidia from eggs that were obtained from experimentally infected hosts. Their chief purpose was to determine the number and location of the germinal cells. Because of limitations of time and material detailed studies were not made on other structures.

All the observations were on living material. Unstained specimens were examined with the oil immersion objective and compared with those on which neutral red and Nile blue sulphate were used as *intra vitam* stains.

The germinal cells in the miracidia of these three species of *Trichobilharzia* were found in rather elongate compact masses extending from just back of the large central nerve mass almost to the posterior end of the body (Figs. 1 2, and 3). The individual cells were crowded together and attached tightly to each other since the masses retained their identity and moved as units in the body cavity during the extension and contraction of the miracidia. In the miracidium of *T. stagnicolae* the mass of germinal cells was observed in some specimens to be divided into two parts (Fig. 3), which, however, were closely attached to each other and moved as a unit. The germinal cells in the miracidium of *T. elvae* were larger and in smaller numbers than in those of the other two species (Fig. 1). They also appeared, at least in some specimens, to be more loosely organized, and they did not extend as far back as in the other two species.

Counts were made of the germinal cells in a small number of miracidia of each species. In 12 miracidia of *T. elvae* the number varied from 10 to 18 with an average of 13; in *T. physellae* five counts varied from 20 to 30 with an average of 26; and in *T. stagnicolae* the numbers in 8 specimens were from 21 to 30 with an average of 22. While it would take a larger series to determine whether the numbers are different in the last two species, it is evident that the smaller number as well as the larger size of the germinal cells in the miracidium of *T. elvae* is a definite specific difference from *T. physellae* and *T. stagnicolae*.

Apparently the only previous description of the germinal cells of the miracidium for a species of the genus *Trichobilharzia* is that given for *T. szidati* by Neuhaus (1952). He described them in this species as present in an oval compact mass which filled a large part of the posterior half of the body. Although he did not give actual counts he showed 13 in his drawing

*A joint contribution from the University of Michigan Biological Station, the School of Hygiene and Public Health of the Johns Hopkins University, and Kansas State College.

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[1]McMullen and Beaver (1945) used the name *T. ocellata* (La Valette, 1855) for this species. Szidat (1942) showed that the cercariae that had been referred to this species by various European workers really belong to several different species. On account of La Valette's very meagre description it cannot be determined with certainty which of them is really *C. ocelatta*. We will, therefore, use the specific name *elvae* which Miller (1923) gave to the cercaria of this species in his original description.

(Fig. 19). This small number and the fact that the mass does not extend nearly to the posterior end suggests a resemblance to *T. elvae.*

The grouping of the germinal cells in *Trichobilharzia* miracidia into a solid elongate mass differs from their arrangement in other schistosomes and related forms. In descriptions and drawings of schistosome miracidia in the literature in which the germinal cells are shown at all clearly they appear as scattered cells in the body cavity, usually with cytoplasmic processes which probably serve to attach them to the body wall and to each other (Price, 1931, Fig. 13; Tang, 1938, Figs. 1 and 2; Wall, 1941, Fig. 19; Singh, 1950, Fig. 4).

A few other points from our observations should be mentioned and can be compared with Neuhaus' (1952) description of the miracidium of *T. szidati.* The miracidia of the three species that we studied appeared to be about the same size. Ten of *T. elvae* which were heat killed, measured 0.102 to 0.153 mm in length (av. 0.122 mm) and 0.044 to 0.057 mm in width (av. 0.048); ten of *T. stagnicolae* treated in the same way varied from 0.102 to 0.166 mm in length (av. 0.133) and from 0.044 to 0.069 mm in width (av. 0.059). The single specimen of the miracidium of *T. physellae* that was measured under slight cover glass pressure after staining with neutral red had a length of 0.121 mm. Neuhaus stated that the free swimming miracidium of *T. szidati* was about 0.18 mm long and 0.050 mm wide. While this is somewhat larger than our measurements, no conclusions can be drawn on the relative size of the miracidia in these four species without having for comparison more numerous measurements made under uniform conditions.

Neither for *T. szidati* nor for our three species was the exact number of ciliated epithelial cells determined. Figures 1, 2, and 3 show that they are arranged in four rows as in other schistosome miracidia (Price, 1931, Fig. 12; Faust and Hoffman, 1934, Fig. 7; Tang, 1938, Fig. 3; Singh, 1950, Fig. 5).

The two median longitudinal rows of relatively large rectangular cells located immediately below the sub-epithelial layer observed by Price (1931, Fig. 14) for *S. douthitti and* Tang (1938, Fig. 4) for *S. japonicum* were seen in the *Trichobiharzia* miracidia (not shown in drawings). Neuhaus did not mention these cells in his description.

In figures 1, 2 and 3 the lateral penetration glands are shown in close contact with the large central glandular structure, the so-called "primitive gut." This central glandular structure was quite large in all our three species and extended beyond the lateral penetration glands to the large central nerve mass. In *T. szidati* Neuhaus described it as much smaller with the lateral glands extending beyond it (Fig. 19). We did not see the two nuclei which he found near the posterior end of this structure.

Neuhaus (1952, p. 25 and Fig. 19) described two large nerves extending forward on each side from the central nerve mass, with short lateral branches connecting with the lateral projections which are characteristic of schistosome miracidia. He considered these lateral projections, which were 0.010 mm long to be sensory papillae (Sinneszapfen). Immediately in front of each of them he found a stiff hair about 0.015 mm in length which he considered also to be sensory. We did not study these structures in detail, but show in the drawings the prominent lateral projections between the first and second row of ciliated epithelial cells. There has been much variation in the descriptions and interpretation of these structures by different workers.

We realize that our studies on the miracidia of the *Trichobilharbia* are of a very preliminary nature. However, they have shown clearly that in the

genus *Trichobilharzia,* as in other schistosomes, there is a considerable multiplication of germinal cells in the miracidium before it penetrates into the intermediate host and metamorphoses into the mother sporocyst.

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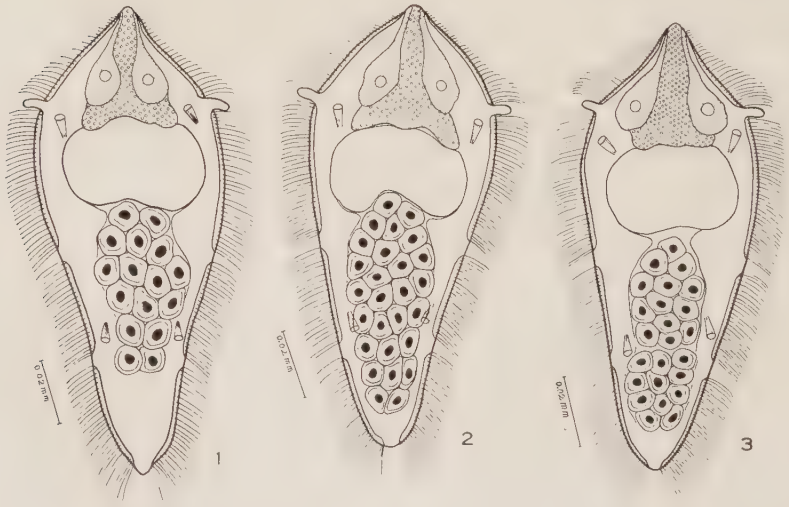

Fig. 1. Miracidium of *Trichobilharzia elvae.*

Fig. 2. Miracidium of *T. physellae.*

Fig 3. Miracidium of *T. stagnicolae.*

Further Studies on the Early Development of the Daughter Sporocysts of *Schistosomatium douthitti**

W. W. CORT, D. J. AMEEL, AND ANNE VAN DER WOUDE

Cort, Ameel and Olivier (1944) reported on the germinal development in the mother and daughter sporocysts of *Schistosomatium douthitti*. They showed that in the early stages of the mother sporocysts the wall grows much more rapidly than the germinal material, producing inflated sacs in which the germinal cells are distributed along the inner surface of the wall. Multiplication of germinal cells is very rapid; the number was estimated to be between 150 and 200 in mothers about 4 days old. In mother sporocysts about a week old the germinal cells begin to develop into daughter sporocyst embryos. Apparently all or almost all of the multiplication of germinal cells occurs before any of them form embryos. The daughter sporocyst embryos remain attached to the wall of the inflated, sausage-shaped mothers until they reach a considerable size and are distinctly elongate. Even in later stages in which many of the daughters are ready to escape they fill only a part of the body cavity of the mother sporocyst. The development of the daughter sporocysts is quite synchronous, although a few germinal cells lag in development and a few embryos are sometimes present in older mothers from which almost all of the daughters have escaped. This produces a situation in which most of the daughter sporocysts in an infection are about the same age.

In the 1944 paper the authors also gave a detailed account of their observations on the germinal development in the daughter sporocysts of *S. douthitti*. Their figures and descriptions showed a very considerable multiplication of germinal cells before any of them started to develop into cercarial embryos. They also suggested that in the older daughter sporocysts there might be a brief phase of multiplication by the formation of germinal masses.

During the summer of 1951 further studies were made on the germinal development in the sporocysts of *S. douthitti*. To obtain the early stages, laboratory raised juveniles of *Stagnicola palustris elodes* (Say) were exposed to miracidia that had hatched from the eggs from experimentally infected white mice. The miracidia were poured into an aquarium containing large numbers of the juveniles. A rather low incidence of infecton was obtained, and many of the positive snails contained only one mother sporocyst. The observations on living material were checked by the study of sections of the mother and daughter sporocysts at different stages of development. Material for sectioning was preserved in position in the snails tissue with Bouin's fixative. The sections were cut 7μ in thickness and stained with Heidenhain's iron haematoxylin.

OBSERVATIONS ON GERMINAL DEVELOPMENT

No attempt was made to obtain the earliest stages of the mother sporocysts. The examination of living material of later stages added very little to the descriptions given by Cort, Ameel, and Olivier (1944). However, some additional data were obtained from sections on the cytology of the germinal cells of the mother sporocysts. One point not clearly brought out in the earlier study was that in many of the mothers the daughter sporocyst em-

*A joint contribution from the University of Michigan Biological Station, the School of Hygiene and Public Health of the Johns Hopkins University, and Kansas State College.

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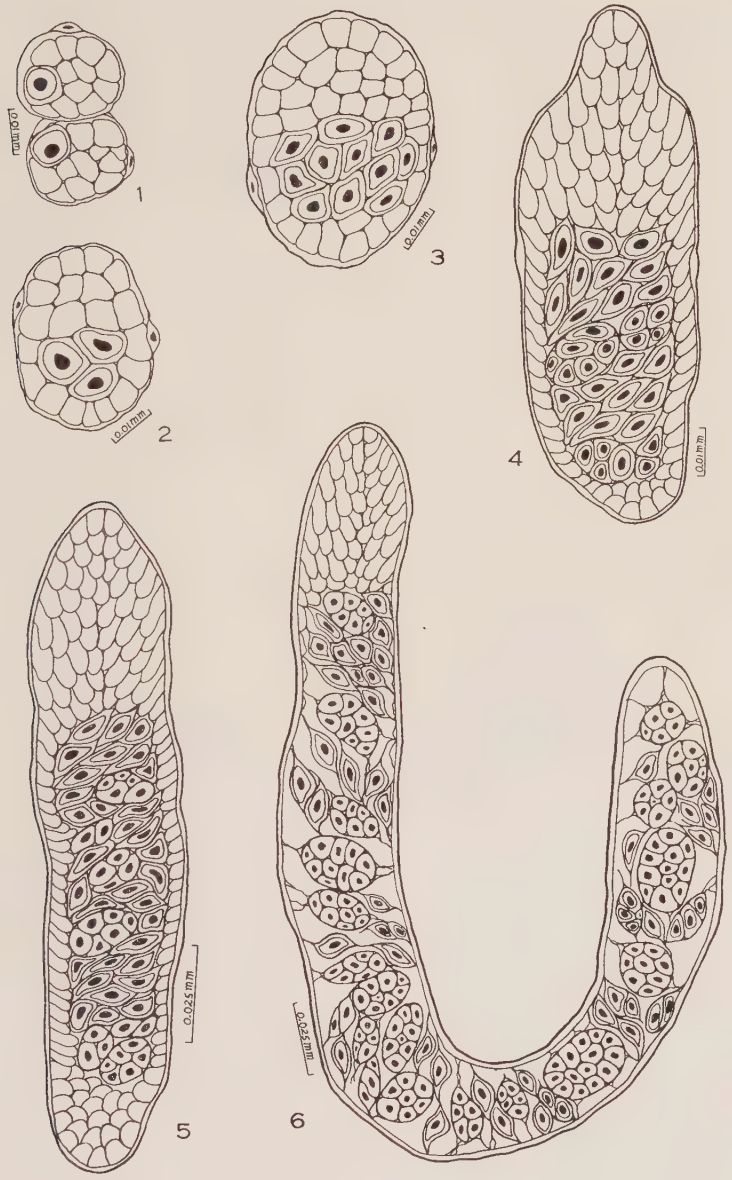
bryos were not evenly distributed along the wall, but were concentrated in certain regions. In some cases also embryos still attached to the wall were attached to each other in groups of two to six or even more. For example, a 15 day old mother sporocyst about 6.5 mm long by 0.25 mm wide contained about 250 embryos, the largest of which was 0.084 by 0.060 mm. In certain regions the embryos were much more numerous than in others and some of them were attached to each other in groups. While most of the embryos were in about the same stage of development a few were composed of only a few cells, and an occasional germinal cell was still present.

This lag in development of a few germinal cells is also shown in figures 7 and 9 which were drawn from sections of mother sporocysts. In figure 7 three germinal cells attached to the wall and to each other are shown close to an attached embryo daughter sporocyst which is just beginning to elongate, and in figure 9 there is a germinal cell attached to the wall in a portion of a mother sporocyst containing a free elongate well-developed daughter. These drawings also show the striking difference in size and structure between the germinal cells and those of the inner lining of the body cavity. Another significant point is the larger size of the germinal cells attached to the wall of the mother sporocyst as compared with those in the daughter sporocysts embryos, which are at a stage where division is constantly taking place.

A detailed description will be given of the development of the germinal material in the early stages of the daughter sporocysts of *S. douthitti* since our observations and interpretations differ in several respects from those of Cort, Ameel and Olivier (1944). The earliest stages of the embryo daughter sporocysts are shown in figures 1, 2 and 3 which were drawn from living material. At these stages the embryos which were still attached to the wall of the mother were surrounded by a thin outer wall containing flattened cells. This enclosed both somatic and germinal cells. In the drawings the soma cells are shown only in outline to contrast them with the germinal cells. In later "germ-ball" stages and in slightly elongate embryos the relations of the somatic cells as the inner lining of the daughter sporocyst wall became evident with the grouping of the germinal cells in the primitive body cavity (Fig. 3). The greater number of somatic cells at one end is significant, since they evidently develop into the "plug" of body wall cells at the anterior end which is so characteristic of later stages. Figure 7 includes a section of a daughter sporocyst embryo at about the same stage as figure 3. In it the differences in size and structure between the somatic and germinal cells can be clearly made out.

In embryo daughter sporocysts about 0.1 mm in length the germinal material had increased considerably in amount and was contained in a clearly defined body cavity (Fig. 4). There were between 20 and 30 germinal elements including a few small embryos. The germinal cells were distinctly elongate and in some of them elongate cytoplasmic processes could be seen connecting them with each other and with the body wall. The inner layer of the body wall was clearly defined and consisted of a single layer of cells except at the anterior and posterior ends. At the anterior end these cells formed a "plug" which extended for more than one-third of the length of the embryo. The thin outer layer of the body wall of the sporocyst was visible at this stage in living material and slight projections indicated the location of the nuclei of its flattened cells. Figure 8 is a section through a daughter sporocyst embryo which appears to be at a slightly earlier stage than figure 4. It was cut at such an angle that the "plug" of body wall cells did not show. Also, the limits of the body cavity could not be made out. However, it



FIGS. 1, 2 and 3. Very early embryos of the daughter sporocysts ("germ ball" stage).

FIG. 4. Daughter sporocyst embryo, 0.12 by 0.04 mm.

FIG. 5. Daughter sporocyst embryo, 0.19 by 0.04 mm. The embryos shown in figures 1 to 5 were taken from mother sporocysts in 23 to 24 day old infections in which no daughter sporocysts were found in the digestive gland.

FIG. 6. Young daughter sporocyst, 0.48 by 0.04 mm, from the digestive gland of a snail with a 28 day old infection.

Germinal development in the sporocysts of *Schisotosomatium douthitti* (continued)

showed the difference in structure between the germinal and body wall cells. Even at this early stage some of the germinal cells had developed into small embryos.

Figure 5 represents a daughter sporocyst embryo at a slightly later stage. The chief change besides an increase in size was an increase in the number of germinal elements. At this stage the germinal cells and youngest embryos were mostly elongate with a horizontal orientation and some showed projections connecting with the body wall. The relations of the germinal material are shown more clearly in figure 10 which is a longitudinal section through a daughter sporocyst embryo at about the same stage as figure 5. In this section the fibrous cytoplasmic extensions from the germinal cells and small embryos could be clearly seen connecting with the wall and with other germinal elements. It appeared as if the germinal elements were arranged in a network of fibers filling the whole body cavity. The anterior "plug" was clearly defined, but the cells of the body wall on account of the thinness of the section did not appear as a continuous layer. However, the cells of the "plug" have the same structure as those of the body wall, and are quite different from the germinal cells. In this section, also, the minute spines at the anterior end were clearly visible. This type of spination is characteristic of the daughter sporocysts of *S. douthitti*. These spines can be seen under high magnification in all stages of elongate sporocysts up to those that have just migrated from the mother. They are not shown in the drawings of the daughters that were made from living specimens, because they are not usually visible at the magnification at which the drawings were made.

In the largest daughter sporocyst embryos still within the mother the total number of germinal elements and the proportion of cercarial embryos had increased. For example, in a daughter sporocyst embryo, 0.25 by 0.05 mm there were about 50 germinal elements, more than one-third of which were small embryos. In another, 0.38 by 0.04 mm, slightly more than 50 germinal elements were counted of which about half were small embryos; the largest was not more than 0.030 mm in diameter. In the largest sporocysts still inside the mother the body cavity was not so crowded, which made it easier to see the attachment of the germinal cells and embryos by cytoplasmic processes to each other and to the body wall. While the anterior "plug" was still quite prominent the thickness of the inner layer of the body wall had become much reduced.

In the migrating stage, in which the daughter sporocysts had escaped from the mother, but had not yet established themselves in the digestive gland of the snail, the most noticeable change was the much greater space between the germinal elements (Fig. 6). There was only a slight increase in the number of germinal elements, about half of which were small embryos. In the sporocyst shown in figure six 28 germinal cells and 32 embryos were counted, the largest being still in the "germ ball" stage and not over 0.040 mm in length. At this stage, the anterior plug was still prominent, but it was not possible in living specimens to see distinctly the inner layer of cells of the body wall.

Observations on the stages after the sporocysts have established themselves in the digestive gland of the snail host agreed very closely with the earlier descriptions (Cort, Ameel and Olivier, 1944, p. 11-13). In older sporocysts the anterior plug was pierced by the birth canal and was reduced to a small amount of tissue on each side of this canal. Although in a few cases embryos were found adhering to each other in small groups, no structures at any stage were seen that could possibly be interpreted as germinal masses.

FIG. 7. Section through a small portion of the body wall of a mother sporocyst showing three germinal cells and an attached embryo.

FIG. 8. Section through a small daughter sporocyst embryo at a slightly earlier stage than figure 4.

FIG. 9. Section through a mother sporocyst showing an elongate daughter sporocyst embryo, and a single large germinal cell attached to the wall.

FIG. 10. Section through a daughter sporocyst embryo about the stage of figure 5. Note the anterior plug of somatic cells and the relations of the germinal elements, both single cells and embryos.

DISCUSSION

The observations recorded above on the germinal development in the daughter sporocysts of *S. douthitti* differ in certain respects from those of Cort, Ameel, and Olivier (1944). Their descriptions and figures of the smallest embryos in the "germ ball" stage show a considerable number of uniform "germ" cells enclosed in a thin membrane. It seems evident that they did not distinguish between the germinal cells and the somatic cells which later form the anterior plug and the inner layer of the wall of the daughter sporocysts. It is significant to point out that these early embryos of *S. douthitti* are like the same stage in strigeoids and plagiorchioids in which also a considerable part of the cells enclosed in the thin outer layer are somatic cells (Van der Woude, Cort, and Ameel, 1952, figs. 4, 5, 6, and 7 and Cort, Ameel, and Van der Woude, 1952, figs. 12, 13, 14 and 15).

Also, the figures and description of the early elongate stages of the daughter sporocysts given by Cort, Ameel, and Olivier in their 1944 paper show large numbers of germinal cells enclosed in a thin outer layer. In our later studies we found in these stages an inner layer of body wall cells which formed a large plug at the anterior end and a much smaller number of germinal elements, some of which had already developed into small embryos (cf. figures 12 and 13 of the 1944 paper with figures 4, 5 and 10 of the present paper). Further, in their descriptions and drawings of the largest daughter sporocysts still within the mother and those in the migrating stage they showed a surprisingly large number of germinal elements (127 to 236), very few of which were embryos (cf. figs. 14 and 15). In fact, they stated that rarely were cercarial embryos present until after the daughters had escaped from the mother. In contrast, our descriptions and drawings of these stages show a large anterior plug, a thin inner layer of the body wall, considerably fewer germinal elements, and a large proportion of cercarial embryos. It would seem that by their methods of examination they had failed to distinguish the somatic cells of the anterior plug and inner layer of the body wall from germinal cells, and had not been able to see the small embryos.

Since two of the workers (Cort and Ameel) were involved in both series of studies on *S. douthitti,* it is possible to make some suggestions in explanation of the differences in observations recorded above. In the first place, the germinal elements in the early stages of the daughter sporocysts of the schistosomes are much more difficult to make out in unstained living material than in most of the forms we have studied. Therefore, in the 1944 studies a great deal of use was made of *intra vitam* staining with neutral red. In more recent work we have found that too heavy staining with neutral red, especially if the preparation is examined too long under the microscope, has a tendency to make it more difficult to distinguish germinal from somantic cells and will sometimes even break up small embryos. In fact, we were able in the summer of 1951 to make preparations that looked like the figures in the 1944 paper by too heavy staining of daughter sporocyst embryos. It seems probable, therefore, that the errors in observation in the earlier studies on the germinal material in the daughter sporocysts of *S. douthitti* were caused by too heavy staining with neutral red and observations on too old preparations. It should be stated that these errors might have been avoided if sections had been made as has been done in all our recent work. We should add also that we have found in recent observations on the development of the daughter sporocysts of *Trichobilharzia stagnicolae* that the same errors were made in the early studies with this species (Cort and Olivier, 1943, figs. 9-11).

The suggestion that was made in the 1944 paper that germinal masses might play a part in the multiplication of germinal material in the daughter sporocysts of *S. douthitti* needs further comment. Cort and Olivier (1943) found numerous groups of germinal elements in the mother and daughter sporocysts of *Trichobilharzia stagnicolae* (Talbot, 1936). They interpreted them as germinal masses similar to those found earlier in strigeoids (Cort and Olivier, 1941). This led to the suggestion that adhering groups of 2 to 5 embryos (Cort, Ameel, and Olivier, 1944, figs. 21-24) which were sometimes found in later stages of the daughter sporocysts of *S. douthitti* might be the remnants of germinal masses. Although sometimes in our present studies we found small groups of adhering embryos in daughter sporocysts, we are convinced that no germinal masses are present in the daughter sporocysts of *S. douthitti,* and that all embryos develop directly from germinal cells. In fact, from recent unpublished studies we now know also that the development of embryos in both mother and daughter sporocysts of *T. stagnicolae* is directly from germinal cells and that the structures that Cort and Olivier (1943) designated as germinal masses were in reality temporary groups of adhering germinal elements.

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Germinal development in the sporocysts of *Schisotosomatium douthitti*

Known and Suspected Plant-parasitic Nematodes of Rhode Island, 1.[1]

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A popular misconception concerning plant-parasitic nematodes in New England has been that they exist in limited numbers, presumably due to the severity of winter temperatures. It has since been found that nematodes apparently cause as much trouble in Rhode Island as in the more temperate areas of the country. Endoparasitic forms such as *Heterodera, Pratylenchus,* and *Meloidogyne* are prevalent, but do not seem to be as troublesome as ectoparasitic forms such as *Tylenchorhynchus, Hoplolaimus,* and possibly *Xiphinema.* A series of papers dealing with the numerous known and suspected plant-parasitic nematodes of Rhode Island will be forthcoming.

(1) *Longidorella parva* Thorne, 1939, a rare Dorylaim suspected of parasitizing greenhouse chrysanthemum roots.

[1]Contribution No. 804 from the Rhode Island Agricultural Experiment Station, Kingston.

Two females and 3 larvae of *L. parva* were isolated from a sample of greenhouse soil containing numerous plant-parasitic and free-living nematodes. This species has hitherto been reported only in cultivated fields in Utah. The soil sample in which it was currently found came from a greenhouse bench in which chrysanthemums (*Chrysanthemum morifolium* Ram.) had grown poorly. Since the mouth parts of this species are similar to those occurring in *Longidorus* and *Xiphinema* which are believed to be plant-parasitic, *Longidorella* likewise is suspected as being plant-parasitic and may have contributed to the poor growth of the plants.

A detailed study of the Rhode Island specimens revealed a few differences when compared to the species described by Thorne (1939) as the type for the genus. The more outstanding of the variations are the presence of lateral body pores in the cervical region and the absence of a constriction separating the anterior portion of the esophagus from the enlarged basal portion. Similarities in appearance and structure are so numerous, however, that one scarcely can doubt that the Rhode Island specimens are identical with the type species.

Longidorella parva Thorne, 1939

Figure 1

Measurements*: 2♀: L=0.65mm. (0.6-0.7mm); a=18.7 (17.8-19.6); b=2.9; c=17.7 (16.8-18.5); V—$^{13}59^{13}$ (58-60).

Body robust and cylindrical, tapering gradually from base of esophageal region to lip region and sharply from anus to terminus. Lips distinct and bearing prominent cephalic papillae. Lip region set-off by slight constriction. Cuticle apparently composed of 2 layers, outer layer bearing extremely faint, fine annulations. Body assuming a ventrally arcuate shape when animal is killed by gradual heat (Fig. 1,A). Amphids stirrup-shaped and elongate, about 5μ wide at head (Figure 1,D). Spear attenuated, about 42μ long, usually arched ventrally. Spear extensions 40μ long, arched ventrally, and joined loosely to spear. Total length of spear and spear extensions about 82μ. Esophagus between base of spear extensions and basal esophageal bulb a slender non-muscular tube. No constriction observed separating anterior portion of esophagus from enlarged basal bulb in 2 females and 3 larvae examined. Nerve ring situated midway between base of spear extensions and beginning of esophageal bulb. Esophageal bulb cylindrical in shape, muscular, 21μ wide, and containing prominent dorsal esophageal gland nucleus. Esophago-intestinal valve bluntly-conical to hemispherical in shape, about 7μ long and 9μ wide. A series of minute, lateral body pores observed along esophageal area. Excretory pore situated in region of nerve ring (Fig. 1,B). Female reproductive organ amphidelphic. Ovaries reflexed about 1/3d their length (Fig. 1,E). Prerectum about 20μ long and 14μ wide. Rectum distinct, about same length as prerectum. Tail ventrally arcuate, elongate-conoid in shape (Fig. 1,C). Males not observed.

Diagnosis—Longidorella with elongate-conical tail and of shorter length than *L. chappuisi* (Schneider, 1935) Thorne, 1939 and *L. pygmaea* (Steiner,

*L = length; a = length divided by greatest width; b = length divided by length of oesophagus; c = length divided by length of tail; V = position of vulva in percentage of body length measured from anterior end, with superior figures indicating maximum extent of anterior and posterior gonads,, respectively, from vulva. In each case, the first figure is the average and the figures in parentheses are the minimum and maximum observed.

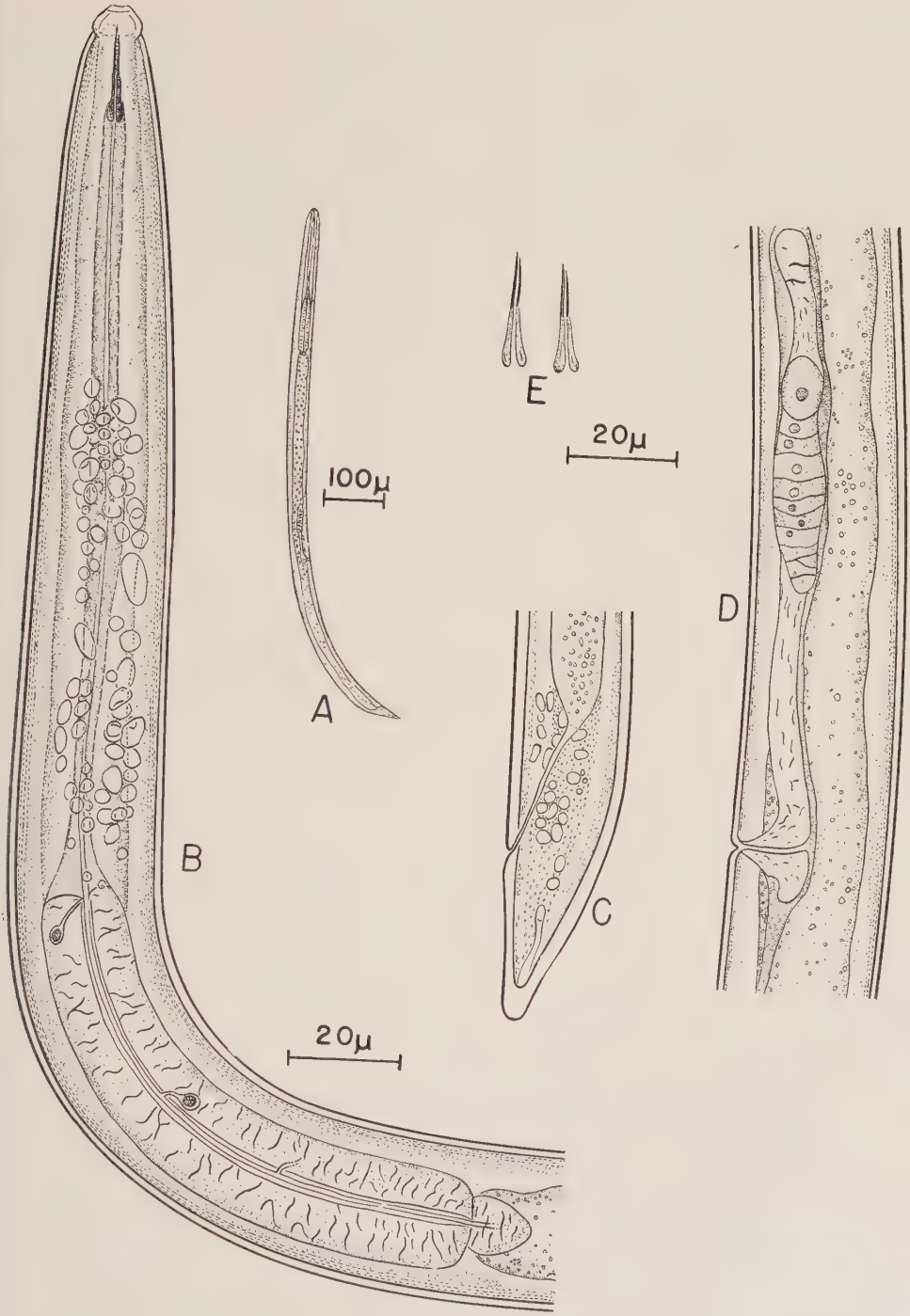

FIG. 1. *Longidorella parva*. A.—Adult female in typical death position when killed by gradual heat. B.—Esophageal region. C.—Posterior end. D.—Amphid showing anterior amphidial pore, median amphidial duct, and posterior amphidial pouch. E.—Reproductive region of body.

1914) Thorne, 1939 which have a blunt conical and hemispherical tail respectively.

Habitat—Cultivated field soil near Salt Lake City and Salem, Utah and greenhouse soil, Allenton, Rhode Island.

(2) *Discomyctus brevicaudatus,* a new species of Dorylaimoidea from boxwood roots and soil.

Twelve females and 4 larvae of this species were found associated with roots of a declining tree boxwood, *Buxus sempervirens* var. *arborescens* L. Although this species clearly belongs to the subfamily *Tylencholaiminae,* Filipjev, it possesses characteristics which relate it to both *Tylencholaimus* and *Discomyctus.* Thorne (1939) separates *Discomyctus* from *Tylencholaimus* in that the former genus has anterior, slender, non-muscular esophageal tube set off from the basal enlarged portion, while the latter has an anterior muscular esophagus which is not set off. On this basis the species in consideration clearly is a *Discomyctus,* since the anterior esophagus is non-muscular and sharply differentiated from the muscular basal portion. Other characteristics which relate it to *Discomyctus* are the small disc-like structure anteriorly surrounding the vestibule and the monodelphic and prodelphic female reproductive organ. The sub-digitate tail shape, however, is at variance with the filiform tail shapes of the other two species of *Discomyctus.*

Key to species of *Discomyctus*

1. Tail dorsally convex and subdigitate ..... *brevicaudatus* n.sp.
   Tail filiform 2
2. Vestibular disc set off by deep constriction ..... *cephalatus* Thorne, 1939
   Vestibular disc set off by depression only ..... *longicaudata* Imamura, 1931

Discomyctus brevicaudatus n.sp.

Figure 2

Measurements*: 12♀ :0.88mm. (0.69-1.03mm); a=29.1 (22.4-34.4); b=3.7 (2.9-4.2); c=26.1 (22.4-28.1); V—[14]62% (60-64%)

Body cylindroid, tapering anteriorly to amalgamated lip region which is set off by slight constriction. Tapering abruptly posteriorly from prerectum to form dorsally convex, subdigitate tail (Fig. 2,C). Cuticle composed of two layers: outer layer finely annulated with striations appearing as single rows of fine punctations; inner layer bearing obliquely longitudinal markings extending from cervical region to tail. Posterior third of body assuming a ventrally arcuate position when animal is killed by gradual heat (Fig. 2,A). Lateral chord 7μ wide midway between posterior esophageal bulb and ovary. Lip region discoid, of 9.0-9.5μ in width, anteriorly bearing obscure disc-like inner papillary circle of 3.0-3.5μ width. Amphids stirrup-shaped, 4μ wide. Buccal stylet weak, 17μ long, composed of anterior sclerotized portion about 6.4μ long and less heavily sclerotized posterior portion (spear extension) of 10.5μ in length (Fig. 2,E). Posteriorly, stylet is trifurcate, bearing delicate off-set knobs of 2μ width. Guiding ring not observed. Esophagus comprised anteriorly of slender, non-muscular, colorless tube of 5μ width separated by slight constriction from enlarged radially-musculated posterior portion of 16μ width (Fig. 2,B). Nerve ring not observed. Lumen of posterior esophageal bulb with heavily sclerotized walls. Dorsal and one ventrosubmedian gland nuclei observed in some specimens. Esophago-intestinal valve bluntly conical, of 13μ width and 16μ length. Intestinal cells colorless, containing

*See footnote, page 50.

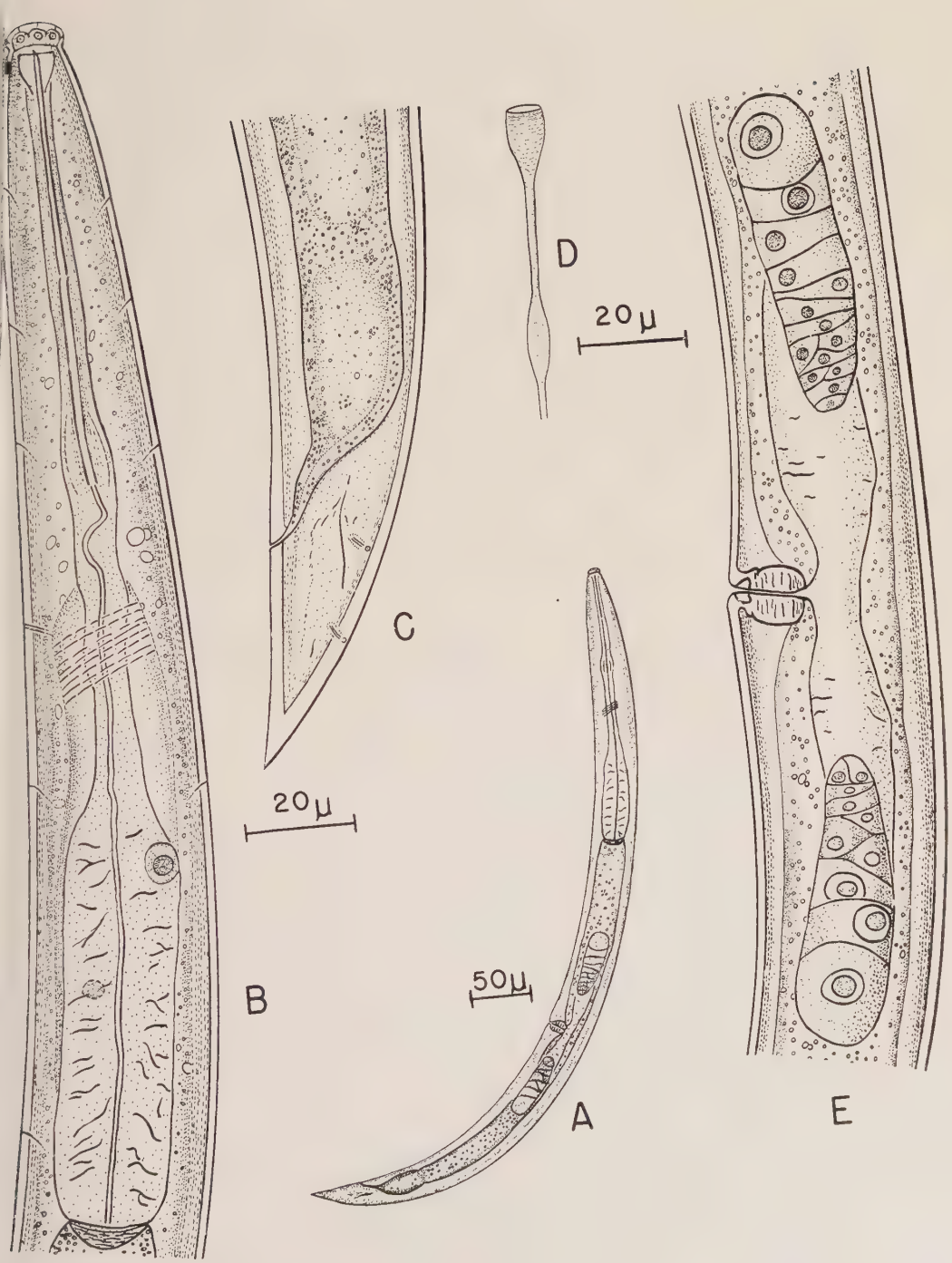

FIG. 2. *Discomyctus brevicaudatus.* A.—Adult female in typical death position when killed by gradual heat. B.—Esophageal region. C.—Posterior end. D.—Reproductive region of body. E.—Buccal stylets.

minute granules. Female reproductive organ monodelphic and prodelphic (Fig. 2,D). Vulva transverse. Reflexed portion of ovary about 52μ long and 8μ wide. Posterior female sexual branch represented by short knob-like projection. Prerectum 5-6 times anal body width. Rectum slightly longer than anal body diameter. Males not observed.

Diagnosis—*Discomyctus* with dorsally convex, subdigitate tail easily distinguished from *D. cephalatus* Thorne, 1939 and *D. longicaudatus* (Imamura, 1931) Thorne, 1939 which possess filiform tails.

Type Locality—Boxwood roots and soil from nursery near Kingston, Rhode Island, U. S. A.

(3) Observations on *Hoplolaimus uniformis* Thorne, 1949, a root parasite of various ornamental plants.

This species, which has been reported only from the Western United States, was found in extremely large numbers around roots of a number of ornamental plants primarily in the eastern part of Rhode Island. In each case roots and soil examined came from plants that were either declining or had died due to "reasons unknown." Among the species of plants thus affected are flowering dogwood (*Cornus florida* L.), pink flowering dogwood (*Cornus florida* var. *rubra* West.), magnolia (*Magnolia soulangeana* Soul.), American arbor-vitae (*Thuja occidentalis* L.), azalea (*Rhododendron* sp.), and box-leaf holly (*Ilex crenata* Thunb.).

A detailed study of 6 males and 6 females resulted in the following formulae:*

6 ♀: 1.6mm (1.3-1.8mm.); a=36.1 (33.8-40.3); b=8.2 (7.3-9.3); c=64.8 (51.6-75.8); $V=^{21}(^{20\text{-}22})\ 55(51\text{-}61)\ ^{20}(^{18\text{-}22})$

6 ♂: 1.3mm (1.2-1.4mm); a=38.1 (34.1-41.5); b=6.5 (4.5-8.4); c=40.1 (35.5-43.5); T=45(37-53)**

Although the above measurements differ somewhat from those given for this species by Thorne (1949), certain important diagnostic features, such as position of the phasmid near the anus, definitely relate the Rhode Island forms to those described by Thorne.

Stylets of mature females examined averaged 48.0μ (46.4-49.9μ) in length and consisted of a heavily sclerotized anterior portion of 24.9μ (21.6-27.6μ) and a less heavily sclerotized posterior portion (stylet extension) of 23.1μ (22.3-24.8μ). Width of the knobbed portion at the base of the stylet was 8.5μ (7.7-9.6μ).

Males had stylets averaging 45.0μ (43.6-46.7μ) in length with an anterior portion of 24.0μ (22.3-25.5μ) and a posterior portion of 21.0μ (19.1-22.7μ). Stylet knobs were 6.5μ (5.3-7.1μ) in width. The orifice of the dorsal esophageal gland was situated 9.1μ (8.8-9.6μ) posterior to the stylet knobs. The length of the male spicule was 41.6μ (38.2-42.8μ) while the distinctive gubernaculum was 21.1μ (19.8-23.4μ) long.

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*See footnote, page 50.

**Average, minimum and maximum extent of testis as measured in percentage of body length from cloaca.

The Identity of *Eimeria arloingi* and *E. faurei* of Sheep and Goats

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At the present time it is commonly accepted that there are at least 5 species of coccidial parasites infecting domestic sheep and goats. The literature on two of these species, *Eimeria arloingi* and *E. faurei,* is rather confusing because of an early view that all coccidia of sheep were *E. faurei* whereas all those of goats were *E. arloingi.* This early view was followed by the subsequent belief that all the coccidia of sheep and goats were of one species, *E. faurei.* Although the oocysts of *E. faurei* can be readily distinguished from those of other coccidia of sheep and goats, there is some question as to whether or not there are one or more other species which have oocysts that may be indistinguishable from those of *E. arloingi.*

The present paper is concerned with an analysis of the literature which pertains especially to the identity of *E. arloingi* and *E. faurei.* Certain findings from studies on experimental infections with these two parasites produced by the present writer (Lotze, 1952) also are included.

Original descriptions of *Eimeria faurei* and *Eimeria arloingi.*

In 1901, Moussu and Marotel described a coccidial parasite of sheep under the name *Coccidium faurei.* Coccidia were reported from sheep prior to this date, but were regarded as identical with those found in rabbits. According to the description of Moussu and Marotel, the oocysts they were describing ranged from 30 to 42 microns in length by 18 to 26 microns in width. They were ovoid to subspherical in shape, and contained a distinct micropyle, 3.5 microns in width, at the smaller pole; no mention was made of the presence or absence of a polar cap. The schizonts were said to be up to 250 to 300 microns in diameter.

In 1905, Marotel described a coccidial parasite of the goat under the name *Coccidium arloingi.* The oocysts were described as ellipsoid in shape, from 25 to 27 microns in length by 16 to 18 microns in width, and possessing a polar cap. Marotel described the schizonts as 18 to 23 microns in diameter. He stated that he regarded *Coccidium faurei* as a *distinct* species.

EARLIER LITERATURE

Baldrey (1906), F. P. Martin (1907), Karsten (1913), Lerche (1920, 1921), Douwes (1920, 1921), Schein (1921), Curasson (1921), and H. M. Martin (1930), reported finding coccodia in sheep and goats and, with few exceptions, either made no attempt to identify the species or assumed that the coccidia of sheep were *E. faurei* and those of goats, *E. arloingi.* Lerche (1920, 1921) stated that the coccidia found by him in the sheep in Germany were *Eimeria arloingi.* Douwes (1920) reported finding coccidia in sheep which had originated in Asia Minor, and expressed the opinion that they were not *E. faurei;* in a later paper, however, Douwes (1921) definitely identified these coccidia as *E. faurei,* but his description fits better that of *E. arloingi.* His illustrations showed that he was dealing probably with mixed infections and that one of the parasites involved was *E. arloingi.*

Nöller, Schürjohann, and Vorbrodt (1922) apparently intended to show that the sheep coccidia, then generally called *E. faurei,* were the same as the goat coccidia, *E. arloingi.* They infected experimentally a "Ziegenlamm," or kid, with oocysts taken from a goat that had died of coccidiosis, and also three sheep with oocysts of unstated origin, but presumably from sheep.

They did not specifically name their "Schafkokzid" or "Ziegenkokzid," but they stated that the oocysts of both possessed a polar cap. Since the oocysts of *E. faurei* do not have a polar cap, it must be presumed that *E. faurei* was not present and that these authors were probably dealing with other oocysts, perhaps those of *E. arloingi.* These workers did not show, as they apparently intended to do that *E. faurei* and *E. arloingi* were identical. Separate reports by Schürjohann (1922) and Vorbrodt (1923) did not give any additional information on this point.

Möller (1923) examined coccidia of wild and domestic sheep and goats in the Zoological Garden in Berlin. He identified coccidia found in goats as *Eimeria arloingi,* and stated that those found in sheep did not differ from those in goats. In spite of this, however, he referred to the coccidia of sheep as *E. faurei.*

Wenyon (1926) erroneously interpreted the findings in Nöller, Schürjohann, and Vorbrodt's paper. On page 844 of his textbook on Protozoology he stated ". . . . it seems not improbable that *E. arloingi* is identical with *E. faurei* of sheep. In fact Nöller, Schürjohann, and Vorbrodt (1922) have conducted cross-infection experiments with sheep and goats, and claim to have established the identity of the two forms." In figure 363 of Wenyon's textbook, the illustration is labelled: *"Eimeria faurei* from the intestine of the goat. (After Nöller, Schürjohann and Vorbrodt, (1922)." As already noted, the German authors did not perform cross-infection experiments, nor did they show that *E. faurei* and *E. arloingi* were identical. In their paper they did not specifically name the coccidia they were dealing with, referring to them only as "Schafkokzid" and "Ziegenkokzid." In view of the fact that *E. faurei* was at that time considered to be "Schafkokzid" and *E. argloingi* the "Ziegenkokzid" it is reasonable to assume that Nöller, Schürjohann and Vorbrodt thought they were dealing with those two species.

In his textbook of Veterinary Parasitology, Marotel (1927) mentioned *E. faurei* and *E. arloingi* as having oocysts with a polar cap. From his description and figure it is clear that he mistook *E. intricata* Spiegl, 1925 for *E. faurei* Moussu and Marotel, 1901.

Yakomoff (1931) discussed and figured coccidia occurring in sheep in Russia. Like the other authors already mentioned, he reported finding *E. faurei,* but his illustrations show that he actually found *E. arloingi.* He also described and figured a new species, *E. aemula,* but his figures suggest that this was *E. faurei.*

More Recent Literature

Balozet (1932) gave an excellent review of the literature on coccidia occurring in sheep and goats. He recognized five species in sheep and goats, namely, *Eimeria faurei,* Moussu and Marotel, 1901; *E. arloingi* Marotel, 1905; *E. intricata,* Spiegl 1925; *E. parva,* Kotlan, Mocsy and Vadja, 1929; and *E. nina-kohl-yakimovi* Yakimoff and Rastegaieva, 1930. Balozet clearly differentiated *E. faurei* and *E. arloingi* from the other species.

Becker (1934) followed Balozet in recognizing five valid species.

Christensen (1938) reported on the oocysts of coccidia found in domestic sheep in the United States. He listed and redescribed the five species mentioned by Balozet, and added two species which he considered to be new, namely, *E. pallida* and *E. granulosa.* According to Christensen, the oocyst of *E. granulosa* has a polar cap, is typically shaped like a broad-shouldered urn, the latter character differentiating this species from all others having a polar cap.

Honess (1942) reported the occurrence of coccidial oocysts in the feces of the Rocky Mountain Sheep, *Ovis canadensis,* and domestic sheep in Wyoming. He found in these animals, all the species recorded by Christensen, except *E. pallida.* He described two new species, *Eimeria ah-sa-ta* and *E. crandallis.* Both of these resemble morphologically *E. arloingi* and fall within the size ranges ordinarily given for that species.

In the 1949 edition of his textbook on Veterinary Parasitology, Marotel reproduced the figures published in his earlier edition, but labeled them *Marotelia faurei* and *Marotelia arloingi,* crediting the generic name *Marotelia* to Ratz (1905). His *Marotelia faurei,* called *Eimeria faurei* in 1927, is *E. intricata* Spiegl, 1925. Marotel stated that *M. arloingi* produced intestinal coccidiosis in sheep and goats. In footnotes he stated that in small ruminants there were some rare *Eimeria* without polar caps, namely, *E. intricata,* ovoid, *E. yakimowi,* ellipsoid, and *E. parva* round. We have been unable to locate the paper by Ratz, 1905, in which, according to Marotel (1949), the generic name *Marotelia* is proposed.

Discussion and Conclusions

The belief that the same species of coccidial parasites occur in sheep and goats is apparently not as yet supported by cross-infection studies. The report of Nöller Schürjohann and Vorbrodt (1922), stated by Wenyon (1926) as having shown that these workers conducted cross-infection studies, does not contain sufficient information for that conclusion.

The writer (1952) produced experimentally pure infections of both *E. arloingi* and *E. faurei* of ovine origin in lambs which had been raised free of parasites, including coccidia. The oocysts shed in the *E. arloingi* infections varied greatly in size and shape, but in no instances were urn-shaped oocysts observed. The oocysts shed in the infections of *E. faurei* were fairly constant in shape and size, and resembled those of no other valid species of coccidial parasite described from sheep or goats.

The writer found the largest or mature schizonts of *E. arloingi* to be about 150 microns in diameter, and those of *E. faurei* about 100 microns in diameter; both schizonts contained thousands of merzoites. From this, it is apparent that the schizont described as 300 microns in diameter by Moussu and Marotel in their original description of *E. faurei* does not belong to that species. Furthermore, the small schizonts described by Nöller, Schürjohann, and Vorbrodt (1922) from infected sheep show that they were dealing with parasites besides *E. arloingi.* Unless the schizont of *E. arloingi* in sheep is different from that in goats, it might be concluded also that the small schizont described for *E. arloingi* by Marotel, in his original description of the parasite, belongs to some other parasite.

According to the information now available, *E. arloingi* and *E. faurei* are distinct species occurring in both sheep and goats. These two species possess oocysts which fit their original descriptions, but the descriptions of the schizonts apparently were not of these two organisms. The evidence for the occurrence of both species in sheep and goats is shown by the similarity of oocysts recovered from these host animals. Additional evidence that *E. arloingi* occurs in both sheep and goats is afforded by an illustration given by Nöller, Schürjohann, and Vorbrodt (1922) of "Kokzidienherde" in the small intestine of goats. Similar structures or groups of swollen villi as shown by these authors occurred in the small intestine of sheep experimentally infected with *E. arloingi* by the present writer.

The question remains as to whether or not there is more than one species of coccidial parasite having oocysts of the *"E. arloingi* type" in sheep and goats. Further studies are necessary to determine the status of *E. ah-sa-ta* and *E. crandallis* of Honess (1942).

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A Modified *en face* Technique

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In making *en face* preparations it is essential that the object being cut be held as immovable as possible. For this purpose a plastic slide was found to be a simple and helpful adjunct. Two grooves were cut in a plastic slide as shown in Fig. 1 A. The longitudinal groove was first scored with a sharp needle and then deepened with a round ended probe. The resulting groove was roughly U-shaped in cross section. Fig. 1 X-X. The transverse cut was made at right angles to the longitudinal one. This transverse groove was made with the instrument to be used in decapitation, in this case a cataract knife, and cut down below the level of the longitudinal groove. Fig. 1 Y-Y. The lateral ends of the transverse groove were widened slightly so that the knife blade could be easily inserted. It was necessary to do this grooving under the dissecting microscope in order to control the width and depth of the grooves. These variables are of course dependent on the particular material to be cut.

Nematodes decapitated using this device were prepared in the manner given by Buhrer (Proc. Helm. Soc. Wash. 16: 3-6). After arranging the nematode in the groove glycerine gelatin was added and allowed to solidify. Under the dissecting microscope the point of the cataract knife was placed in the slightly widened end of the transverse groove. Since at the junction of the two grooves the transverse groove is only slightly wider than the knife, it is held firmly in line while a downward pressure accomplishes the beheading. Further details of the processing follow Buhrer (*loc. cit.*). The holder may be cleaned by immersion in hot water (60-70C) followed by a brief rinse in 95 per cent ethyl alcohol.

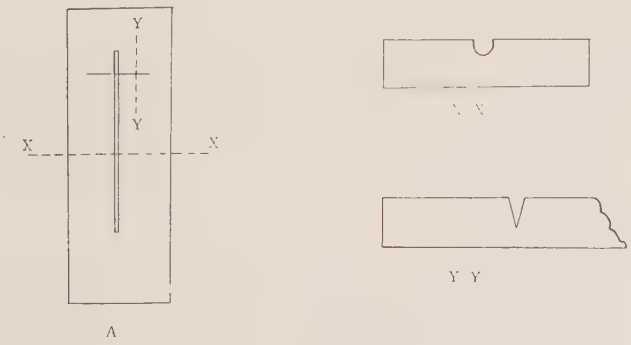

Fig. 1 A.—Slide (½ natural size)
X-X.—Cross section (2X)
Y-Y.—Long. section (2X)

Isospora jeffersonianum n. sp. from the Blue-spotted Salamander, *Ambystoma jeffersonianum* (Green), and *Eimeria grobbeni* Rudovsky, 1925 from the California Newt, *Triturus torosus* (Rathke)

DAVID J. DORAN*

Although there have been no *Isospora* reported from salamanders (order Caudata), Walton (1941) lists 9 species from frogs and toads (order Salientia). Of the 9 species of *Eimeria* from amphibians listed by Hardcastle (1943), only *Eimeria ranarum* (Labbé, 1894) Doflein, 1099, found in *Ambystoma opacum* by Rankin (1937), is known to occur in North America.

The writer has examined the following salamanders for coccidia: 28 *Triturus torosus* (Rathke), 65 *Batrachoseps attenuatus attenuatus* (Eschscholtz), 4 *Batrachoseps attenuatus pacificus* (Cope), 7 *Aneides lugubris lugubris* (Hallowell), 7 *Ambystoma jeffersonianum* (Green) and 3 *Ambystoma tigrinum tigrinum* (Green). Two *Ambystoma jeffersonianum* harbored what appears to be a new species of *Isospora*. Three *Tritirus torosus* were parasitized by an *Eimeria* which the writer considers as *Eimeria grobbeni* Rudovsky, 1925.

Isospora jeffersonianum n. sp.

(Fig. 1)

DIAGNOSIS: (All measurements are in microns) Oocysts spherical, 18.5 to 22.5 in diameter. Oocyst wall smooth, 1.5 thick, apparently composed of two layers. A small knob, 1.5 to 2.0 wide (possibly a micropyle) present. Sporocysts ellipsoidal, 15.5 to 16.0 long by 7.0 to 8.0 wide, with a knob at one end. Sporocyst residual body spherical, highly granular, 2.0 to 2.7 in diameter. Sporozoites cresent-shaped, 6.0 to 7.0 long, with nuclei equi-distant between the ends. Sporulation time (interval between deposition of a one-hour fecal sample by the host and first appearance of sporulated oocysts), 64 to 72 hours.

HOST: *Ambystoma jeffersonianum* (Green).

LOCALITY: Birchmont Road, near Bemidji, Beltrami County, Minnesota.

DISCUSSION: This species differs from 8 of the species reported from frogs and toads with respect to oocyst dimensions. *Isospora wladimirovi* Yakimoff, 1930, described from the European tree toad, *Hyla arborea*, measures 18 to 25 microns by 15.5 to 21.22 microns and, therefore, bears the closest resemblance to *I. jeffersonianum*. Since *I. jeffersonianum* differs from 8 of the species with regard to measurements and since *I. wladimirovi* is ellipsoidal rather than circular, designation as a new species seems justified.

Eimeria grobbeni Rudovsky, 1925

(Fig. 2)

Slightly ellipsoidal oocysts, measuring 11 to 13 microns long by 10 to 12 microns wide, were found in the feces of 3 *Tristurus torosus* collected in Fish Canyon, San Bernardino Mountains, Los Angeles County, California. The measurements and general morphology closely resemble *E. grobbeni* which

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was described from a European newt, *Salamandra atra*. Rudovsky (1925) states that *E. grobbeni* oocysts measure 9 to 10 microns by 10 to 11 microns. The oocysts from the California newt may belong to a new species. However, due to the close similarity of oocysts from two such closely related genera as *Salamandra* and *Triturus*, it seems best at present to consider them as oocysts of *Eimeria grobbeni* Rudovsky, 1925.

SUMMARY

Isospora jeffersonianum n. sp. is described from the blue-spotted salamander, *Ambystoma jeffersonianum* (Green) collected from northern Minnesota. *Eimeria grobbeni* Rudovsky, 1925 is reported from the California newt, *Triturus torosus* (Rathke) collected from southern California.

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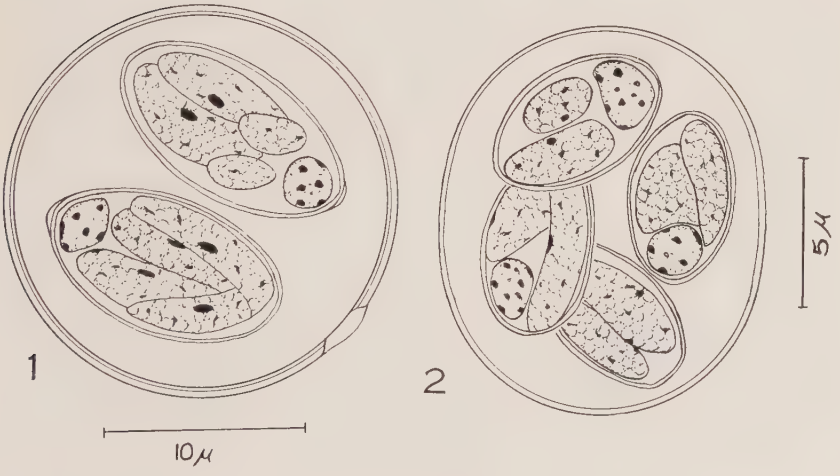

Fig. 1.—Mature oocyst of *Isospora jeffersonianum* n. sp.

Fig. 2.—Mature oocyst of *Eimeria grobbeni* Rudovsky, 1925.

Observations on *Heterodera weissi* Steiner, 1949 (Heteroderidae, Nematoda)

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Heterodera weissi Steiner, 1949, a parasite of knotweed, *Polygonum pensylvanicum* L., is known to occur in a number of states east of the Rocky Mountains. During the summer of 1950 specimens in all stages of development were observed infecting the type host at the Plant Industry Station at Beltsville, Maryland. Infected plants showed no apparent symptoms of decline although they bore, in most cases, rather heavy nematode populations. The present work was undertaken for the purpose of amplifying the original diagnosis with measurements and photomicrographs of various stages in the life cycle.

Measurements of eggs indicated that average length and width varied between cysts. Sixty eggs (ten taken from each of 6 cysts) were found to average 97.7μ (82.9μ — 105.5μ) in length and 46.0μ (42.05μ — 50.9μ) in width. Table 1 shows the maximum, minimum, and average egg measurements from each cyst examined. All of these eggs contained larvae apparently in the second stage of development (Fig. 1, A). A molt within the egg has been reported for other species in the genus.

Measurements of second-stage larvae were obtained by employing the method used by Raski (1950). A coverslip was placed over eggs immersed in a drop of 4 per cent formalin on a microscope slide. Gentle pressure applied to the coverslip released many of the larvae from enclosing egg shells without damaging the nematodes. Sixty larvae, taken at random from numerous cysts, averaged 405μ (280μ — 445μ) in length with buccal stylets 21.4μ (20.0μ — 24.1μ) in length. The hyaline portion of the tail of these larvae, i.e., the portion of the tail posterior to the granular material of the body cavity, (Fig. 1, B) had an average length of 21.4μ (18.4μ — 24.0μ).

The average length of 25 males was 999.8μ (895.0μ — 1121.0μ) while their buccal stylets averaged 24.7μ (22.0μ — 26.0μ). The length of the spicules was 33.5μ (30.0μ — 41.0μ), measured in a straight line from tip to tip.

Measurements of 50 mature female cysts were found to average 541μ (421μ — 676μ) by 348μ (218μ — 541μ).

It appears quite likely that *H. weissi* develops in a fashion similar to that of *H. schachtii* Schmidt (Raski 1950) and *H. rostochiensis* Wollenweber (Chitwood and Buhrer 1946). Photomicrographs of various stages in its development were made from roots stained by the lactophenol-acid fuchsin method of McBeth, Taylor, and Smith (1941). Figure 1, C shows a larva, apparenty in the late second or early third stage of development, with head embedded in the stele of a root. A larva, apparently in the third stage is shown in Figure 1, D, while Figure 1, E shows a female larva apparently in the fourth stage with body almost rupturing the root epidermis. Males were observed (Fig. 1, F) immediately underlying the root epidermis, in a folded position as illustrated by Steiner (1949).

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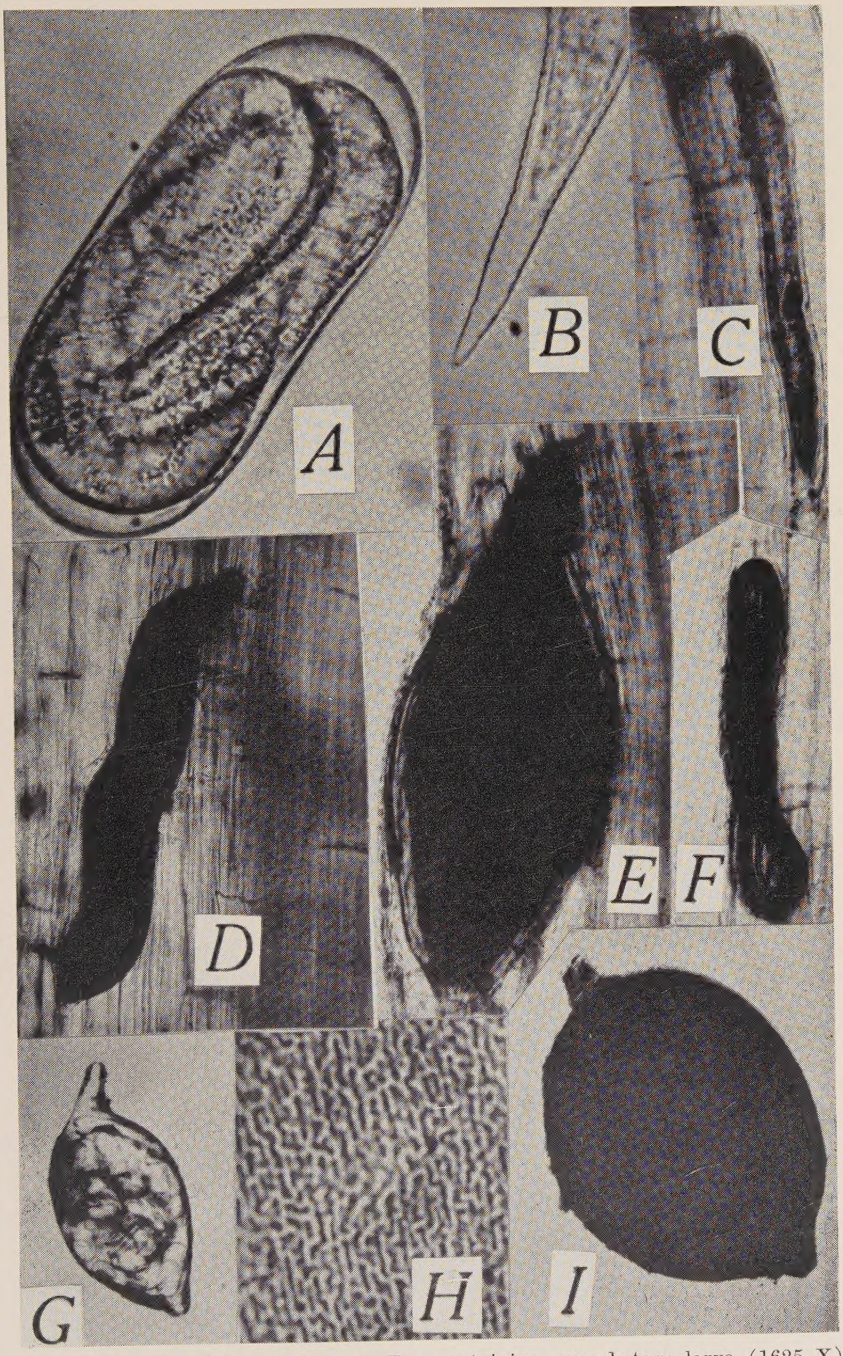

FIG. 1.—*Heterodera weissi*. A. Egg containing second-stage larva (1625 X). B. Non-protoplasmic portion of second-stage larval tail (1000 X). C. Late second-stage or early third-stage larva with head in vascular tissues of root (200 X). D. Third-stage larva (150 X). E. Fourth-stage larval female (175 X). F. Adult male in cortex of root (175 X). G. Young adult female (100 X). H. Characteristic markings on cyst wall (925 X). I. Mature cyst (100 X).

Young adult females (Fig. 1, G) were of an opaque, whitish color and were considerably smaller than mature cysts. Such specimens showed little evidence of bearing markings on the cyst wall characteristic of mature cysts (Fig. 1, H). Steiner (1949) describes these markings appearing as "a network of transverse, meandering and anastomosing ridges, separating depressions . . . mostly elongate, somewhat rhomboidal in shape." Mature cysts (Fig. 1, I) appeared amber to dark brown in color.

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TABLE 1

MAXIMUM, MINIMUM AND AVERAGE MEASUREMENTS OF 10 EGGS FROM EACH OF 6 CYSTS OF *Heterodera weissi*

Cyst No.	Egg length in μ			Egg width in μ		
	Maximum	Minimum	Average	Maximum	Minimum	Average
1	104.0	98.2	101.1	50.5	43.6	48.1
2	96.3	82.9	90.8	44.4	40.6	42.9
3	102.0	93.6	98.3	48.0	43.2	46.4
4	104.0	97.8	100.8	45.1	40.2	43.2
5	97.8	93.2	96.3	50.9	41.7	57.6
6	105.5	94.8	99.6	49.4	41.5	47.6

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